

Review

Cannabidiol Signaling in the Eye and Its Potential as an Ocular Therapeutic Agent

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Key Words

Cannabidiol • Molecular target • Mechanism of action • Therapeutic potential • Ocular pharmacology

Abstract

Cannabidiol (CBD), the major non-intoxicating constituent of *Cannabis sativa*, has gained recent attention due to its putative therapeutic uses for a wide variety of diseases. CBD was discovered in the 1940s and its structure fully characterized in the 1960s. However, for many years most research efforts related to cannabis derived chemicals have focused on Δ^9 -tetrahydrocannabinol (THC). In contrast to THC, the lack of intoxicating psychoactivity associated with CBD highlights the potential of this cannabinoid for clinical drug development. This review details *in vitro* and *in vivo* studies of CBD related to the eye, the therapeutic potential of cannabidiol for various ocular conditions, and molecular targets and mechanisms for CBD-induced ocular effects. In addition, challenges of CBD applications for clinical ocular therapeutics and future directions are discussed.

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Introduction

A brief history of cannabis

Cannabis sativa is a plant species that includes both cannabis and hemp. It first appeared in Chinese medical texts around 2000 years ago [1]. Records from Britain indicate that cannabis was brought from Egypt by Napoleon's troops in the early 1800s [2]. Shortly thereafter, hemp was introduced to Western medicine when in 1840, a hemp tincture from ground plant matter was reported to be an effective treatment for convulsive disorders and tetanus [3]. By 1851, a cannabis extract was included in the 3rd edition of the United States Pharmacopoeia and readily available in American pharmacies [4, 5].

In 1913, however, cannabis was made illegal in California due to a wide-spread anti-narcotics campaign [5]. Cannabis became federally illegal when Harry Anslinger from California introduced the Marijuana Tax Act of 1937 banning the sale and use of cannabis

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nationally [5, 6]. A negative stigma continued to develop in the US around cannabis, then associated with narcotics, that culminated with the Controlled Substances Act (CSA) of 1970, which classified cannabis and cannabinoids as Schedule I with no recognized medical use [7]. Recently, America is witnessing a revival in the popularity of cannabis, both medically and recreationally. In 1996, California was the first state to legalize cannabis for medical use and more states have followed California in recent years [8]. To date, 16 states and Washington D.C. have legalized both medical and recreational cannabis with an additional 26 states legalizing medical cannabis at varying degrees. Moreover, the Agricultural Acts of 2014 and 2018 removed hemp from the list of controlled substances and redefined industrial hemp as cannabis containing less than 0.3% THC [9, 10]. As a result of the recent wave of recreational and medical cannabis legalization, in conjunction with the end to the prohibition of hemp, cannabis research is quickly expanding.

Cannabidiol

Cannabidiol (CBD) is one of over 120 chemicals produced by the *Cannabis sativa* plant termed phytocannabinoids [11, 12]. There are potentially more, as 21 previously unknown cannabinoids were recently identified [13]. The two most abundant phytocannabinoids in cannabis are psychoactive and intoxicating Δ^9 -tetrahydrocannabinol (THC) and non-intoxicating CBD.

CBD was first isolated in the 1940 and its structure and stereochemistry fully determined in 1963 [14, 15]. CBD and THC are both derived from cannabigerolic acid [16]. Although the structure of CBD was discovered before THC [15, 17], THC had been the major focus of research related to cannabis and cannabinoids. This focus is driven, in part, by the activity of THC at the canonical cannabinoid receptors, CB1 and CB2. However, there are many targets for cannabinoids other than CB1 and CB2. For example, CBD has upwards of 65 known targets consisting of receptors, enzymes, ion channels and transient receptor potential (TRP) channels [18].

Cannabinoids in pharmaceuticals

Cannabinoid containing drugs are approved for medical use in the USA and other countries. The drugs differ in their formulation and indicated uses. Dronabinol (Marinol) was the first cannabinoid-containing medicine approved by the FDA in 1985. It is a soft gel capsule containing synthetic THC [19]. Syndros is an oral solution of dronabinol [20]. Cesamet (nabilone) is the third synthetic cannabinoid drug approved by the FDA in May of 2006 [21]. All three are prescribed for anorexia associated with weight loss in AIDS patients and nausea/vomiting in cancer patients [19-22]. While plant-derived THC is a Schedule I substance, Marinol is listed under Schedule III and Cesamet and Syndros are controlled under Schedule II [19-21].

Epidiolex is an oil formulation of CBD approved by the FDA in June of 2018 for treatment of Lennox-Gastaut syndrome and Dravet syndrome, two rare and severe forms of pediatric epilepsy [23]. In July of 2020, it was approved for treating seizures in a rare genetic disease, tuberous sclerosis complex (TSC) [24]. Epidiolex is the only FDA approved drug containing a compound directly derived from cannabis. It was originally classified as schedule V, but is no longer a controlled substance as the FDA deemed it safe and effective for treatment of the aforementioned conditions [25]. Sativex is a 1:1 alcohol solution of THC and CBD administered as an oromucosal spray that is approved in 25 countries for the treatment of pain and spasticity in multiple sclerosis patients [26]. Despite its approval in other countries, Sativex is not yet approved by the FDA in the US.

Research on cannabidiol

CBD, through a variety of mechanisms and targets, has numerous potential therapeutic uses for a plethora of conditions. The assertion of potential therapeutic actions of CBD is based on pre-clinical data, limited clinical data and ongoing human clinical trials. Pre-clinical studies show that CBD has antioxidant [27, 28] anti-inflammatory [27], anti-

convulsant [29, 30], neuroprotective [31], and anti-cancer properties [32]. CBD also shows potential as a therapeutic agent in cardiovascular [33], neurological, and neuropsychiatric disorders [26]. The completed clinical trials involve CBD use in epilepsy and seizures disorders (21 trials), general pain and pain associated disorders (19 trials), drug abuse and use disorders (14 trials), other neurologic conditions (4 trials) and psychiatric conditions (11 trials). In addition, there are currently 85 active clinical trials in the United States containing CBD (including Epidiolex and Sativex) on clinicaltrials.gov.

Over the past two decades, multiple studies have investigated the therapeutic potentials of CBD in the eye. There are several published reviews of cannabinoids for treatment of glaucoma [34, 35], and retinal disorders [36, 37]. Nevertheless, there are currently no reviews that focus solely on CBD for ocular conditions. In this review, we aim to fill the gap in literature with a focus on CBD ocular pharmacology. We will discuss therapeutic potentials of CBD for ocular conditions, ocular molecular targets for CBD, and mechanisms of actions of CBD in the eye.

Results

Therapeutic potentials of cannabidiol for ocular conditions

CBD is recognized for its antioxidant, anti-inflammatory and neuroprotective properties. In this section, we discuss the observed effects of CBD in ocular tissues and its indication for ocular disorders. Specifically, we will discuss studies of CBD in corneal inflammation and pain, endotoxin-induced inflammation, excitotoxicity, diabetic retinopathy, and intraocular pressure (Table 1 and Table 2).

Corneal Inflammation and pain

The cornea is a thin and avascular tissue that is innervated by sensory nerves. When corneal damage occurs due to infection, surgery, or trauma, it can develop into corneal neuropathic pain characterized by hyperalgesia, chronic and debilitating pain, and inflammation [38, 39]. The inflammatory response to corneal damage leads to the production of proinflammatory cytokines, recruitment of leukocytes, release of pain-producing neuropeptides, and neovascularization (NV) in the cornea [38, 39].

In a recent study, CBD was found to be anti-nociceptive and anti-inflammatory in a mouse model of corneal hyperalgesia [39] (Table 1). Mice with silver nitrate cauterized corneas that treated with CBD showed lower pain scores in capsaicin pain challenges, indicating an antinociceptive effect of CBD. Moreover, CBD treated corneas showed less corneal neutrophil infiltration which is indicative of a CBD-induced anti-inflammatory effect. Lastly, WAY100635, a 5HT_{1A} antagonist, blocked the effects of CBD, suggesting that the anti-inflammatory and anti-nociceptive effects are likely mediated through activation of the serotonin 5HT_{1A} receptor [39]. This study highlights CBD as a potential therapeutic for corneal pain and inflammation.

Table 1. Therapeutic potentials of CBD for ocular conditions

Ref.	Disease	Model	CBD route	CBD effect	Therapeutic relevance
[39]	Corneal pain and inflammation	Silver nitrate cauterization-induced corneal hyperalgesia in mice	topical	↓corneal hyperalgesia ↓neutrophil infiltration	CBD is anti-nociceptive and anti-inflammatory in the cornea following corneal surface injury
[44]	Retinal inflammation	Endotoxin-induced inflammation in rat retina and primary retinal microglial cells	intraperitoneal	↓ adenosine reuptake ↓ TNF α production	CBD is anti-inflammatory in the retina via inhibiting adenosine reuptake
[51]	Retinal neurotoxicity	Intravitreal injection of NMDA in rats	intravenous	↓ nitrotyrosine formation ↓ nitrite/nitrate ↓ lipid peroxidation ↓ apoptosis	CBD is neuroprotective against retinal excitotoxicity
[58]	Diabetic retinopathy	Streptozotocin-induced diabetic rats	intraperitoneal	↓ TNF α , ICAM-1, and VEGF expression ↓ p38 MAP kinase activation ↓ ROS formation	CBD protects retina from diabetes related inflammation, vascular permeability, and neurotoxicity

Table 2. The effects of CBD on intraocular pressure

Ref	Species	Route	Dose Frequency	Vehicle	Dose	Effect on IOP
[73]	Rabbit	intravenous	1 application	2% Tween 60 and 3% Arlacel in water	1 mg/kg 10 mg/kg	No change No change
[70]	Monkey	Oral	1 application	2% Tween 60 and 3% Arlacel in water	10 mg/kg	No change
[71]	Rabbit	intravenous	1 application	100% alcohol	0.1 mg/animal 1 mg/animal 10 mg/animal	No change No change No change
[72]	Rabbit	intravenous	1 application	25% BSA in 95% EtOH	1 mg/animal	No change
[75]	Rabbit	Topical	1 application	Mineral oil	0.0001%	↓
					0.001%	↓
					0.01%	↓
					0.1%	↓
					1%	↓
				Sesame oil	0.1%	No change
[74]	Human	intravenous	1 application	25% human serum albumin	20 mg/person	↓
[76]	Cats	Topical via minipump	Continuously for 9 days	Polyethylene glycol	20 mg/hour	↓
[64]	Human	Sublingual	4 spray at 5-minute intervals	Oromucosal spray with non-specified vehicle	20 mg 40 mg	No change Transient ↑
[63]	Wild type C57/B6 Mice	topical	1 application	Tocrisolve, a soya-based solvent	5 mM	↑ at 1 and 4 hours
	CB1 knockout mice				5 mM	↓ at 1 hour

Endotoxin-induced inflammation

The mammalian retina contains three distinct glia cells types: Müller cells, astrocytes, and microglia. Microglial cells are the resident macrophages of the retina and play important roles in retinal homeostasis [40]. Activation of microglial cells induces the release of pro-inflammatory cytokines, such as IL-1 β and TNF α , instigating an inflammatory response. Prolonged microglial activation and chronic inflammation contribute to disease pathology and retinal degeneration [40].

The degree of microglial activation may relate to the severity of injury. *In vitro* and *in vivo* treatment with lipopolysaccharide (LPS), an endotoxin from bacteria, is used to study inflammation through activated microglia [41]. Extracellular adenosine can function as an endogenous anti-inflammatory agent suppressing immune cell responses. For example, adenosine inhibits pro-inflammatory cytokine expression such as TNF α [42]. However, the anti-inflammatory effects of adenosine are short, as it is rapidly taken up by adjacent cells. Inhibitors of adenosine uptake may enhance the adenosine signaling and endogenous activity [43]. CBD has been shown to decrease TNF α expression and inhibit equilibrative nucleoside transporter 1 (ENT1) reuptake of adenosine in LPS treated primary microglia cells and retinas from LPS treated rats [44] (Table 1). The effect of CBD is primarily mediated through the activation of the A_{2A} receptor, the most abundant adenosine receptor in the rat retina, as a result of CBD-induced inhibition of adenosine reuptake [44]. These results suggest that CBD may be a good anti-inflammatory agent for endotoxin-induced retinal damage.

Excitotoxicity

Excitotoxicity is implicated in glaucoma as a result of elevated levels of the excitatory neurotransmitter glutamate in the retina [45, 46]. Over-stimulation of a glutamate receptor, such as the N-methyl-D-aspartate (NMDA) receptor, a sodium and calcium permeable channel, results in excess intracellular calcium. Increased intracellular calcium is cytotoxic, as well as induces release of more glutamate, cellular swelling, and eventually cell death [47, 48]. The process of excitotoxicity also involves the activation of nitric oxide (NO) synthase and accumulation of NO and superoxide. Overproduction of these oxygen species produces oxidative stress leading to lipid peroxidation, mitochondrial dysfunction, DNA damage and eventually, cell death [46, 49].

One method to measure oxidative stress is through peroxynitrite/nitrotyrosine formation and lipid peroxidation [50]. Peroxynitrite is a product of a superoxide reaction primarily responsible for oxide- and superoxide-dependent cytotoxicity. It is highly unstable, highly reactive and difficult to measure, therefore the presence of peroxynitrite is measured by levels of nitrotyrosine which is formed by nitration of protein-bound tyrosine [50].

In a rat model of neurotoxicity, intravitreal injection of NMDA induces nitrite/nitrate accumulation, lipid peroxidation, nitrotyrosine production, apoptosis, and inner retinal neuronal loss [51]. CBD treatment decreased levels of peroxynitrite/nitrotyrosine production, prevented neurotoxicity, and lowered the amount of apoptosis (Table 1). The neuroprotective effect of CBD was dependent upon blockage of nitrotyrosine formation [51]. The retinal antioxidant and neuroprotective effects of CBD in the rat model of retinal excitotoxicity suggest that it may be beneficial as a neuroprotectant for the treatment of ocular conditions such as glaucoma.

Diabetic retinopathy

Globally, diabetic retinopathy is a major cause of vision loss. Oxidative stress, caused by reactive oxygen species, is one of the main factors in diabetic retinopathy progression [52]. The retina is particularly sensitive to reactive oxygen species because it is the most metabolically active tissue in the body and therefore easily affected by diabetes [52]. Diabetic retinopathy is characterized by retinal hypoxia, increased retinal vascular permeability, and retinal angiogenesis [52, 53]. These processes cause the death of inner retinal and ganglion cells and ultimately, vision loss.

Inflammation is another important component in diabetic retinopathy. Hyperglycemia triggers the release of proinflammatory cytokines such as vascular endothelial growth factor (VEGF), Intercellular adhesion molecule-1 (ICAM-1), and Tumor necrosis factor α (TNF α) [54, 55]. The elevation of these proinflammatory cytokines further facilitates pathologic changes in diabetic retinopathy as a result of neovascularization by VEGF, leukocyte adhesion and transmigration by ICAM-1 and further release of cytokines by TNF α [53-55]. Research shows that VEGF, ICAM-1, and TNF α are upstream regulators of proinflammatory and oxidative stress pathways which activate p38 MAP kinase [55]. Activation of p38 MAP kinase has been reported in diabetic retinas in high glucose conditions and is implicated in retinal ganglion cell apoptotic death [56-58]. In addition, p38 MAP kinase activity is linked to vascular hyperpermeability in diabetic retinas [55].

One report assessed the therapeutic potential of CBD in a streptozotocin-induced diabetic rat model through measurement of oxidative stress and proinflammatory cytokines [58] (Table 1). The antibiotic streptozotocin (produced by *Streptomyces achromogenes*) induces type 1 diabetes through partial destruction of the pancreatic β cells after a single injection. In the streptozotocin-induced diabetic rat model there were increases in oxidative stress, retinal neuronal cell death, and vascular hyperpermeability associated with increased levels of VEGF, ICAM-1, TNF α , and activation of p38 MAP kinase [58]. Importantly, CBD decreased reactive oxygen species (ROS) formation, suppressed VEGF, ICAM-1, and TNF- α expression, and prevented activation of p38 MAP kinase [58]. Taken together, these findings suggest that CBD is a potential therapeutic agent for diabetic retinopathy capable of protecting against inflammation, retinal neuronal cell death, and preservation of the blood-retinal barrier.

Intraocular pressure

An estimated 3 million Americans have glaucoma, a major cause of irreversible blindness with no cure [59, 60]. Even with therapeutic intervention, approximately 10% of those diagnosed still experience vision loss [59, 60]. Although not always elevated, intraocular pressure (IOP) is currently the only treatable factor of the disease. Drug therapies such as prostaglandin analogs, β -adrenergic antagonists, cholinergic agonists, α_2 -adrenergic agonists, and carbonic anhydrase inhibitors are used either independently or in combination, to reduce ocular pressure [61, 62]. In 2018, the FDA approved Rhopressa, a Rho kinase inhibitor, as a novel IOP-lowering drug [61, 62]. These IOP-lowering drugs work to decrease aqueous humor production in the ciliary body and/or increase aqueous humor drainage through the trabecular meshwork or the uveoscleral pathway [61, 62]. For patients that do not respond to the above drugs or drug combinations, or patients developed tolerance to existing drugs, novel medications are needed to lower IOP and to prevent future optic nerve damage and vision loss associated with glaucoma.

THC is well documented and is consistently shown to decrease IOP [63-69]. However, the effect of CBD on IOP is much more controversial (Table 2). So far, nine independent reports have published regarding the effects of CBD on IOP: Four reports indicate that CBD has no effect on IOP [70-73], three reports demonstrate that CBD decreases IOP [74-76], and two reports show an CBD-induced increase in IOP [63, 64].

A recently published study in mice showed an increase in IOP at 1 and 4 hours post topical application of CBD at a 5 mM dose [63]. Interestingly, CBD significantly decreased IOP 1 hour post treatment in CB1 knockout mice and the effect is attributed to GPR18 activation [63]. This article was cited by the American academy of ophthalmology with the headline “CBD oil may worsen glaucoma” [77]. Altogether, the literature does not conclusively show whether CBD increases, decreases, or causes no change to IOP.

Molecular targets and mechanisms for CBD-induced ocular effects

CBD has numerous targets in different categories such as G protein-coupled receptors, enzymes, nuclear receptors, ligand-gated ion channels, transient receptor potential (TRP) channels, and potentially others [18]. Many of these CBD targets are expressed in the eye. This section of the review focuses on the effects of CBD on these molecular targets in the eye (Table 3). It is important to point out that systemic CBD administration may result in CBD metabolites that act through molecular mechanisms different from those of CBD itself.

Serotonin receptor

Thapa et al. showed that CBD can reduce the pain score and neutrophil infiltration in mice after corneal cauterization and capsaicin challenge and this effect is mediated, in part, by 5-HT_{1A} agonism [39] (Table 3). The hypoalgesic and anti-inflammatory effects of CBD seen in wild-type mice were still present in CB2 knockout mice, as well as CB2 knockout mice pretreated with AM251, a CB1 selective antagonist. These results suggest that the hypoalgesic and anti-inflammatory effects of CBD are not mediated by CB1 or CB2 receptors. Moreover, the effects of CBD were blocked in wild-type mice treated with WAY100635, a 5-HT_{1A} receptor antagonist. These data demonstrate that the effect of CBD on corneal hyperalgesia inflammation is mediated by 5-HT_{1A} agonism [39]. In support of the findings of Thapa et al. in the eye [39], CBD has been shown to be a 5-HT_{1A} agonist in other tissues as well [78, 79].

Equilibrative nucleoside transporter 1 and A_{2A} adenosine receptor

CBD has been shown to inhibit TNF- α response to LPS stimulation by inhibiting adenosine reuptake in retinal microglia via adenosine equilibrative nucleoside transporter 1 (ENT1) [44] (Table 3). Cells that were pre-treated with CBD showed inhibition of LPS-induced release of TNF- α . The inhibition of TNF- α release was not further enhanced nor inhibited by pretreatment of NBMPR, an ENT1 selective inhibitor [44]. These results suggest that CBD

Table 3. Molecular targets and mechanisms for CBD-induced ocular effects

Ref	Target	Tissue/ Cells	CBD route	CBD effect	CBD mechanism
[39]	5-HT _{1A}	Mouse cornea	topical	↓ pain score ↓ neutrophil infiltration	5-HT _{1A} agonist, effect blocked by 5-HT _{1A} antagonist WAY100635
[44]	ENT1 A _{2A}	Rat retinal microglia	intraperitoneal	↓ adenosine reuptake ↓ TNF α expression	ENT1 inhibitor, reuptake inhibition blocked by ENT1 inhibitor NBMPR Activate A _{2A} indirectly, effect blocked by A _{2A} antagonist ZM241385
[63]	GPR18 CB1	Wild-type and CB1 knockout mice	topical	↑ IOP in wild-type mice ↓ IOP in CB1 knockout mice	CB1 negative allosteric modulator GPR18 agonist, effect blocked by GPR18 antagonist 01918
[92]	GPR55	Mouse retinal explant cultures	intravitreal	↓ growth cone size ↓ filopodia number ↑ chemorepulsion	GPR55 antagonist, effects absent in GPR55 knockout mice
[98]	TRPV2	Porcine RPE cells	In vitro	↑ intracellular Ca ²⁺	TRPV2 agonist, effect reduced by TRPV2 blocker SKF-96365
[99]	TRPV2	ARPE-19 cells	In vitro	↑ current density ↑ membrane conductance	TRPV2 agonist, effect blocked by TRPV2 blocker SKF-96365

competes with NBMPR for ENT1. Furthermore, CBD inhibited TNF- α in the presence of A_{1A} adenosine antagonist CPX, whereas the effect of CBD was blocked by pre-treatment with A_{2A} adenosine receptor antagonist ZM241385. When CBD and adenosine were co-administered, TNF- α release was greatly reduced showing a synergistic effect that is greater than when either compound was administered alone [44]. In sum, Liou et al. showed that CBD inhibits adenosine reuptake through ENT1, which indirectly causes the enhanced activation of A_{2A} adenosine receptor and reduction of TNF- α release [44]. The effects of CBD on ENT1 and adenosine receptors are corroborated by reports in rat and mouse striatal terminals [80] and in EOC-20 murine microglial cells [81].

CB1 and GPR18

CB1 is a well-established cannabinoid receptor and CBD has been shown to be a negative allosteric modulator of CB1 [82]. CB1 is expressed in the anterior of the eye in the ciliary and corneal epithelium and trabecular meshwork, as well as the posterior of the eye in the retina [83, 84]. GPR18 is a recently identified putative cannabinoid receptor and researchers have shown that GPR18 is activated by N-arachidonoyl glycine, a carboxylic metabolite of the endocannabinoid anandamide [85]. GPR18 was further characterized in 2012 when anandamide and THC, in addition to N-arachidonoyl glycine, were shown to stimulate GPR18-mediated ERK1/2 phosphorylation [86]. Furthermore, CBD was shown to be a biased agonist for GPR18 in 2014 [87]. GPR18 is widely expressed in the ocular tissues, specifically in the ciliary and corneal epithelium, trabecular meshwork, and retina [88, 89].

To date a single paper has reported on the effect of CBD at both CB1 and GPR18 receptors in the eye (Table 3). Miller et al. showed that CBD increases IOP in wild-type mice but decreases IOP in CB1 knockout mice [63]. No CBD effect on IOP was seen in CB1 knockout mice pretreated with O-1918, a GPR18 antagonist. This report highlights that CBD has independent actions both on CB1 as a negative allosteric modulator to raise IOP and on GPR18 as an agonist to lower IOP [63].

GPR55

GPR55 is an orphan receptor activated by lysophosphatidylinositol (LPI) [90]. GPR55 is frequently referred to as a putative cannabinoid receptor because it is activated by phytocannabinoids, endocannabinoids, and synthetic cannabinoids [91]. CBD has been shown to be a GPR55 antagonist [91].

One group studied the involvement of GPR55 in the retina during development [92]. Growth cones are regions of developing neurites which facilitate axon growth by extending actin filaments into filopodia. Filopodia guide the growth cone in response to chemical or electrical stimulus. Cherif et al. found that GPR55 is expressed in growth cones during development, and its activity regulates morphology and growth [92]. Mouse embryonic neurons from GPR55 knockout mice showed smaller growth cones, fewer filopodia, and decreased outgrowth compared to neurons from wild type mice. Furthermore, retinal ganglion cells from wild type mice treated with GPR55 agonists LPI and O-1602 showed increased growth cone size and filopodia number and demonstrated chemoattraction. In contrast, CBD, a GPR55 antagonist, decreased growth cone size and filopodia number, and induced chemorepulsion (Table 3). GPR55 ligands had no effects in embryonic neurons from GPR55 knockout mice [92]. These data suggest that CBD inhibits growth cone activity and axonal growth in the retina in this experimental model.

TRPV Channels

Transient receptor potential (TRP) ion channels are trans-membrane proteins involved in a wide range of chemical and physical sensations including smell, taste, vision, temperature, and pressure [93]. CBD has been shown to be an agonist of TRPV 1, 2, 3, 4 and TRPA1 [94, 95]. TRPV channels are implicated in the activation and desensitization of inflammatory processes and chronic pain [96, 97]. Therefore, CBD may be a desirable therapeutic for chronic pain because it can activate and desensitize the TRPV channels [94, 95].

One group investigated the calcium influx activity of TRPV2 channel activity in porcine retinal pigment epithelial (RPE) cells [98]. They found that CBD strongly increased intracellular Ca^{2+} levels (Table 3). In the presence of TRPV2 channel inhibitor SKF96365, CBD-mediated Ca^{2+} intracellular increase was partially blocked [98]. These data suggest that CBD modulation of Ca^{2+} involves TRPV2, as well as other TRPV channels that are not blocked by SKF96365.

Another study looked at TRPV2 channel regulation in ARPE-19, a human RPE cell line [99]. ARPE-19 cells preincubated with CBD demonstrated a 3-fold increase in current density, an effect that was blocked by SKF96365 (Table 3). CBD also increased membrane conductance and TRPV2 surface expression. TRPV2 are heat sensitive ion channels and heat further increased the CBD mediated increase in membrane conductance. Furthermore, the PI3 kinase inhibitor LY294002 abolished the effect of CBD on membrane conductance and surface expression. These data led to the conclusion that CBD acts through activation of TRPV2 and a PI3 kinase dependent pathway to increase cell surface expression of TRPV2 channels [99].

Discussion

Challenges of using CBD as an ocular therapeutic agent

There are several challenges for practical applications of CBD as an ocular therapeutic agent. Some of these challenges include poor bioavailability, difficulty in topical delivery, and short duration of action.

Bioavailability

An FDA approved drug containing CBD is administered orally [23]. However, oral administration is inefficient due to poor bioavailability of CBD. Low bioavailability of CBD requires that it to be administered at high doses to achieve therapeutic effects. However, a consequence of high dosing is an increase in adverse side effects [100, 101]. The potential adverse effects of CBD include drowsiness, dry mouth, reduced appetite, nausea, and gastrointestinal issues. The most notable serious adverse side effects of CBD are abnormal liver function tests (elevated liver enzymes) [102].

One major factor contributing to the poor bioavailability of CBD is its extensive first pass metabolism [100, 101]. Another factor limiting CBD bioavailability is its hydrophobicity. The chemical structure of CBD contains aromatic rings and an aliphatic side chain, which make it a highly hydrophobic molecule. The hydrophobicity of CBD limits its solubility in water and makes diffusion across aqueous barriers a rate limiting step for diffusion and absorption [100, 101].

Topical delivery

Therapeutic treatments for ocular conditions are frequently administered orally or topically to the eye. Extensive first pass metabolism of CBD prevents a significant amount of drug reaching the eye from oral administration. Therefore, topical administration of CBD is desired.

Developing an ocular topical delivery system is a difficult task. The eye contains sophisticated protective mechanisms and physical barriers to prevent foreign material from entering, which includes the multi-layered cornea and pre-corneal tear film. The alternating lipophilic and hydrophilic nature of the cornea makes ocular drug delivery exceptionally difficult. As a result, less than 5% of drugs applied topically enter the eye [103-105].

CBD is highly hydrophobic and insoluble in water. Studies applying CBD topically used non-aqueous vehicles for delivery [39, 63, 75]. Previously, CBD was topically delivered to the eye in mineral oil, sesame oil, soybean oil, and a soya oil/water emulsion, Tocrisolve [39, 63, 75]. In one report, CBD delivered in mineral oil produced an IOP-lowering effect whilst the effect is absent in sesame oil [75]. With Tocrisolve as a vehicle, Miller et al. [63]

demonstrated that a high dose of CBD increases IOP in wildtype mice, but decreases IOP in CB1 knockout mice. This indicates that at large doses CBD produces off-target effects which are detrimental. Since CBD has at least 65 targets [18], off-target effects of CBD at high doses are very likely. These results highlight a critical need for a vehicle with high ocular permeation to administer CBD in a therapeutically relevant dose.

Duration of action

Another difficulty associated with CBD as an ocular therapeutic is its short duration of action, e.g., in lowering IOP. One report indicated CBD decreased IOP 1-2 hours after topical application to rabbit eyes, and IOP-lowering effects of CBD lasted for up to 5 hours after intravenous administration [75]. In another study, CBD required constant infusion via minipump to induce a decrease in IOP [76]. A short duration of action implies that CBD needs to be applied multiple times throughout the day to maintain therapeutic effects. However, patient compliance will be worsened with frequent dosing. In contrast, greater patient compliance is observed in prescribed medications with once daily dosing [106].

Future directions

So far, CBD has been studied preclinically for its therapeutic potentials in glaucoma, diabetic retinopathy, and corneal injury. Considering its anti-inflammatory, antioxidant, and neuroprotective properties, in the future it would be worthwhile to explore the potential of CBD in treating other ocular conditions, such as uveitis and age-related macular degeneration.

It is important to elucidate the mechanisms of action of CBD in the eye. As highlighted in this review, there are multiple molecular targets of CBD in the eye. Understanding which targets are responsible for the therapeutic and adverse effects of CBD is critical for its effective and safe use as an ocular therapeutic agent.

Finally, in the future, solving the puzzles of dosing and proper formulation for efficient, prolonged topical delivery will usher CBD forth for numerous potential ocular therapeutic indications.

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Statement of Ethics

The authors have no ethical conflicts to disclose.

Disclosure Statement

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References

- 1 Brand EJ, Zhao Z: Cannabis in Chinese Medicine: Are Some Traditional Indications Referenced in Ancient Literature Related to Cannabinoids? *Front Pharmacol* 2017;8:108.
- 2 Piomelli D: The molecular logic of endocannabinoid signalling. *Nat Rev Neurosci* 2003;4:873-884.
- 3 O'Shaughnessy WB, Calcutta MD: New remedy for tetanus and other convulsive disorders. *Boston Med Surg J* 1840:153-155.
- 4 Convention USP: Pharmacopoeia of the United States, ed 3. Philadelphia, PA, Lippincott, Grambo & Company, 1851.
- 5 Gieringer DH: The Origins of Cannabis Prohibition in California. *Contemp Drug Probl* 1999;26:237-288.
- 6 Marijuana Tax Act of 1937. URL: <https://www.druglibrary.org/schaffer/hemp/taxact/mjtaxact.htm>.
- 7 Controlled Substances Act, 1970. URL: <https://www.govinfo.gov/content/pkg/STATUTE-84/pdf/STATUTE-84-Pg1236.pdf>.
- 8 Compassionate Use Act of 1996. URL: https://leginfo.ca.gov/faces/codes_displaySection.xhtml?awCode=HSC§ionNum=11362.5.
- 9 Agricultural Act of 2014. URL: <https://www.congress.gov/bill/113th-congress/house-bill/2642>.
- 10 Agriculture Improvement Act of 2018. URL: <https://www.congress.gov/bill/115th-congress/house-bill/2>.
- 11 Turner CE, Elsohly MA, Boeren EG: Constituents of Cannabis sativa L. XVII. A review of the natural constituents. *J Nat Prod* 1980;43:169-234.
- 12 ElSohly AM, Gul W: Constituents of Cannabis Sativa; in Pertwee R (ed): *Handbook of Cannabis*. Oxford, UK, Oxford University Press, 2014.
- 13 Mudge EM, Murch SJ, Brown PN: Chemometric Analysis of Cannabinoids: Chemotaxonomy and Domestication Syndrome. *Sci Rep* 2018;8:13090.
- 14 Adams R, Hunt M, Clark JH: Structure of Cannabidiol, a Product Isolated from the Marijuana Extract of Minnesota Wild Hemp. I. *J Am Chem Soc* 1940;62:196-200.
- 15 Mechoulam R, Shvo Y, Hashish. I. The structure of cannabidiol. *Tetrahedron* 1963;19:2073-2078.
- 16 Taura F, Morimoto S, Shoyama Y: Purification and characterization of cannabidiolic-acid synthase from Cannabis sativa L.. Biochemical analysis of a novel enzyme that catalyzes the oxidocyclization of cannabigerolic acid to cannabidiolic acid. *J Biol Chem* 1996;271:17411-17416.
- 17 Gaoni Y, Mechoulam R: Isolation, Structure, and Partial Synthesis of an Active Constituent of Hashish. *J Am Chem Soc* 1964;86:1646-1647.
- 18 Ibeas Bih C, Chen T, Nunn AV, Bazet M, Dallas M, Whalley BJ: Molecular Targets of Cannabidiol in Neurological Disorders. *Neurotherapeutics* 2015;12:699-730.
- 19 FDA: MARINOL (dronabinol): Highlights of Prescribing Information, 2017. URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/018651s029lbl.pdf.
- 20 FDA: Syndros: Highlights of Prescribing Information, 2017. URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2017/205525s003lbl.pdf.
- 21 FDA: Cesamet (nabilone) Capsules For Oral Administration, 2006. URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2006/018677s011lbl.pdf.
- 22 Badowski ME, Yanful PK: Dronabinol oral solution in the management of anorexia and weight loss in AIDS and cancer. *Ther Clin Risk Manag* 2018;14:643.
- 23 FDA: Epidiolex: Highlights of Prescribing Information, 2018. URL: https://www.accessdata.fda.gov/drugsatfda_docs/label/2018/210365lbl.pdf.
- 24 FDA: FDA Approves New Indication for Drug Containing an Active Ingredient Derived from Cannabis to Treat Seizures in Rare Genetic Disease, 2020. URL: <https://www.fda.gov/news-events/press-announcements/fda-approves-new-indication-drug-containing-active-ingredient-derived-cannabis-treat-seizures-rare#:~:text=Today%2C%20the%20U.S.%20Food%20and,year%20of%20age%20and%20older>.
- 25 Establishment of a New Drug Code for Marijuana Extract, 2016. URL: <https://www.federalregister.gov/documents/2016/12/14/2016-29941/establishment-of-a-new-drug-code-for-marijuana-extract>.
- 26 Barnes MP: Sativex®: clinical efficacy and tolerability in the treatment of symptoms of multiple sclerosis and neuropathic pain. *Expert Opin Pharmacother* 2006;7:607-615.
- 27 Atalay S, Jarocka-Karpowicz I, Skrzydlewska E: Antioxidative and Anti-Inflammatory Properties of Cannabidiol. *Antioxidants (Basel)* 2019;9:21.

- 28 Hampson AJ, Grimaldi M, Lolic M, Wink D, Rosenthal R, Axelrod J: Neuroprotective antioxidants from marijuana. *Ann N Y Acad Sci* 2000;899:274-282.
- 29 Paolino MC, Ferretti A, Papetti L, Villa MP, Parisi P: Cannabidiol as potential treatment in refractory pediatric epilepsy. *Expert Rev Neurother* 2016;16:17-21.
- 30 Leo A, Russo E, Elia M: Cannabidiol and epilepsy: Rationale and therapeutic potential. *Pharmacol Res* 2016;107:85-92.
- 31 Campos AC, Fogaça MV, Sonogo AB, Guimaraes FS: Cannabidiol, neuroprotection and neuropsychiatric disorders. *Pharmacol Res* 2016;112:119-127.
- 32 Kis B, Ifrim FC, Buda V, Avram S, Pavel IZ, Antal D, Paunescu V, Dehelean CA, Ardelean F, Diaconeasa Z, Soica C, Danciu C: Cannabidiol-from Plant to Human Body: A Promising Bioactive Molecule with Multi-Target Effects in Cancer. *Int J Mol Sci* 2019;20
- 33 Stanley CP, Hind WH, O'Sullivan SE: Is the cardiovascular system a therapeutic target for cannabidiol? *Br J Clin Pharmacol* 2013;75:313-322.
- 34 Passani A, Posarelli C, Sframeli AT, Perciballi L, Pellegrini M, Guidi G, Figus M: Cannabinoids in Glaucoma Patients: The Never-Ending Story. *J Clin Med* 2020;9:3978.
- 35 Pena J, Jimenez C, Schmidt J: Do cannabinoids play a role in the control of glaucoma? *Medwave* 2018;18:e7144.
- 36 Schwitzer T, Schwan R, Angioi-Duprez K, Giersch A, Laprevote V: The Endocannabinoid System in the Retina: From Physiology to Practical and Therapeutic Applications. *Neural Plast* 2016;2016:2916732.
- 37 Kokona D, Georgiou PC, Kounenidakis M, Kiagiadaki F, Thermos K: Endogenous and Synthetic Cannabinoids as Therapeutics in Retinal Disease. *Neural Plast* 2016;2016:8373020.
- 38 Belmonte C, Acosta MC, Merayo-Llones J, Gallar J: What Causes Eye Pain? *Curr Ophthalmol Rep* 2015;3:111-121.
- 39 Thapa D, Cairns EA, Szczesniak AM, Toguri JT, Caldwell MD, Kelly MEM: The Cannabinoids Delta(8) THC, CBD, and HU-308 Act via Distinct Receptors to Reduce Corneal Pain and Inflammation. *Cannabis Cannabinoid Res* 2018;3:11-20.
- 40 Rashid K, Akhtar-Schaefer I, Langmann T: Microglia in Retinal Degeneration. *Front Immunol* 2019;10:1975.
- 41 Wang AL, Albert C, Lau LT, Lee C, Tso MO: Minocycline inhibits LPS-induced retinal microglia activation. *Neurochem Int* 2005;47:152-158.
- 42 Hasko G, Pacher P, Vizi ES, Illes P: Adenosine receptor signaling in the brain immune system. *Trends Pharmacol Sci* 2005;26:511-516.
- 43 Noji T, Takayama M, Mizutani M, Okamura Y, Takai H, Karasawa A, Kusaka H: KF24345, an adenosine uptake inhibitor, suppresses lipopolysaccharide-induced tumor necrosis factor-alpha production and leukopenia via endogenous adenosine in mice. *J Pharmacol Exp Ther* 2002;300:200-205.
- 44 Liou GI, Auchampach JA, Hillard CJ, Zhu G, Yousufzai B, Mian S, Khan S, Khalifa Y: Mediation of cannabidiol anti-inflammation in the retina by equilibrative nucleoside transporter and A2A adenosine receptor. *Invest Ophthalmol Vis Sci* 2008;49:5526-5531.
- 45 Dreyer EB: A proposed role for excitotoxicity in glaucoma. *J Glaucoma* 1998;7:62-67.
- 46 Dreyer EB, Zurakowski D, Schumer RA, Podos SM, Lipton SA: Elevated glutamate levels in the vitreous body of humans and monkeys with glaucoma. *Arch Ophthalmol* 1996;114:299-305.
- 47 Choi DW: Glutamate neurotoxicity and diseases of the nervous system. *Neuron* 1988;1:623-634.
- 48 Waxman EA, Lynch DR: N-methyl-D-aspartate receptor subtypes: multiple roles in excitotoxicity and neurological disease. *Neuroscientist* 2005;11:37-49.
- 49 Coyle JT, Puttfarcken P: Oxidative stress, glutamate, and neurodegenerative disorders. *Science* 1993;262:689-695.
- 50 Misko TP, Highkin MK, Veenhuizen AW, Manning PT, Stern MK, Currie MG, Salvemini D: Characterization of the cytoprotective action of peroxynitrite decomposition catalysts. *J Biol Chem* 1998;273:15646-15653.
- 51 El-Remessy AB, Khalil IE, Matragoon S, Abou-Mohamed G, Tsai NJ, Roon P, Caldwell RB, Caldwell RW, Green K, Liou GI: Neuroprotective effect of (-)Delta9-tetrahydrocannabinol and cannabidiol in N-methyl-D-aspartate-induced retinal neurotoxicity: involvement of peroxynitrite. *Am J Pathol* 2003;163:1997-2008.
- 52 Li C, Miao X, Li F, Wang S, Liu Q, Wang Y, Sun J: Oxidative Stress-Related Mechanisms and Antioxidant Therapy in Diabetic Retinopathy. *Oxid Med Cell Longev* 2017;2017:9702820.
- 53 Aiello LP, Wong JS: Role of vascular endothelial growth factor in diabetic vascular complications. *Kidney Int Suppl* 2000;77:S113-119.

- 54 Kamiuchi K, Hasegawa G, Obayashi H, Kitamura A, Ishii M, Yano M, Kanatsuna T, Yoshikawa T, Nakamura N: Intercellular adhesion molecule-1 (ICAM-1) polymorphism is associated with diabetic retinopathy in Type 2 diabetes mellitus. *Diabet Med* 2002;19:371-376.
- 55 Semeraro F, Cancarini A, dell'Omo R, Rezzola S, Romano MR, Costagliola C: Diabetic Retinopathy: Vascular and Inflammatory Disease. *J Diabetes Res* 2015;2015:582060.
- 56 Igarashi M, Wakasaki H, Takahara N, Ishii H, Jiang ZY, Yamauchi T, Kuboki K, Meier M, Rhodes CJ, King GL: Glucose or diabetes activates p38 mitogen-activated protein kinase via different pathways. *J Clin Invest* 1999;103:185-195.
- 57 Purves T, Middlemas A, Agthong S, Jude EB, Boulton AJ, Fernyhough P, Tomlinson DR: A role for mitogen-activated protein kinases in the etiology of diabetic neuropathy. *FASEB J* 2001;15:2508-2514.
- 58 El-Remessy AB, Al-Shabrawey M, Khalifa Y, Tsai NT, Caldwell RB, Liou GI: Neuroprotective and blood-retinal barrier-preserving effects of cannabidiol in experimental diabetes. *Am J Pathol* 2006;168:235-244.
- 59 Phu J, Agar A, Wang H, Masselos K, Kalloniatis M: Management of open-angle glaucoma by primary eye-care practitioners: toward a personalised medicine approach. *Clin Exp Optom* 2021;104:367-384.
- 60 Jonas JB, Aung T, Bourne RR, Bron AM, Ritch R, Panda-Jonas S: Glaucoma. *Lancet* 2017;390:2183-2193.
- 61 Shalaby WS, Shankar V, Razeghinejad R, Katz LJ: Current and new pharmacotherapeutic approaches for glaucoma. *Expert Opin Pharmacother* 2020;21:2027-2040.
- 62 Lu LJ, Tsai JC, Liu J: Focus: Drug Development: Novel Pharmacologic Candidates for Treatment of Primary Open-Angle Glaucoma. *Yale J Biol med* 2017;90:111-118.
- 63 Miller S, Daily L, Leishman E, Bradshaw H, Straiker A: Delta9-Tetrahydrocannabinol and Cannabidiol Differentially Regulate Intraocular Pressure. *Invest Ophthalmol Vis Sci* 2018;59:5904-5911.
- 64 Tomida I, Azuara-Blanco A, House H, Flint M, Pertwee RG, Robson PJ: Effect of sublingual application of cannabinoids on intraocular pressure: a pilot study. *J Glaucoma* 2006;15:349-353.
- 65 Crawford WJ, Merritt JC: Effects of tetrahydrocannabinol on arterial and intraocular hypertension. *Int J Clin Pharmacol Biopharm* 1979;17:191-196.
- 66 Cooler P, Gregg JM: Effect of delta-9-tetrahydrocannabinol on intraocular pressure in humans. *South Med J* 1977;70:951-954.
- 67 Colasanti BK, Powell SR, Craig CR: Intraocular pressure, ocular toxicity and neurotoxicity after administration of delta 9-tetrahydrocannabinol or cannabichromene. *Exp Eye Res* 1984;38:63-71.
- 68 Fischer KM, Ward DA, Hendrix DV: Effects of a topically applied 2% delta-9-tetrahydrocannabinol ophthalmic solution on intraocular pressure and aqueous humor flow rate in clinically normal dogs. *Am J Vet Res* 2013;74:275-280.
- 69 Purnell WD, Gregg JM: Delta(9)-tetrahydrocannabinol, euphoria and intraocular pressure in man. *Ann Ophthalmol* 1975;7:921-923.
- 70 Waller CW, Benigni DA, Harland E, Bedford JA, Murphy JC, ElSohly MA: Cannabinoids in Glaucoma III: The Effects of Different Cannabinoids on Intraocular Pressure in the Monkey, in Agurell S, et al (eds): *The Cannabinoids: Chemical, Pharmacologic, and Therapeutic Aspects* 1984, pp 871-880.
- 71 Liu JH, Dacus AC: Central nervous system and peripheral mechanisms in ocular hypotensive effect of cannabinoids. *Arch Ophthalmol* 1987;105:245-248.
- 72 Green K, Symonds CM, Oliver NW, Elijah RD: Intraocular pressure following systemic administration of cannabinoids. *Curr Eye Res* 1982;2:247-253.
- 73 ElSohly MA, Harland EC, Benigni DA, Waller CW: Cannabinoids in glaucoma II: the effect of different cannabinoids on intraocular pressure of the rabbit. *Curr Eye Res* 1984;3:841-850.
- 74 Perez-reyes M, Wagner D, Wall ME, Davis KH: Intravenous administration of cannabinoids and intraocular pressure, in: Braude, MC and Szara, S (eds): *The Pharmacology of Marihuana*, Raven, New York, 1976, pp. 829-832.
- 75 Green K, Wynn H, Bowman KA: A comparison of topical cannabinoids on intraocular pressure. *Exp Eye Res* 1978;27:239-246.
- 76 Colasanti BK, Brown RE, Craig CR: Ocular hypotension, ocular toxicity, and neurotoxicity in response to marihuana extract and cannabidiol. *Gen Pharmacol* 1984;15:479-484.
- 77 Shelton B: CBD Oil May Worsen Glaucoma. *American Academy of Ophthalmology*, 2019. URL: <https://www.aao.org/eye-health/news/cbd-oil-may-worsen-glaucoma>.
- 78 Russo EB, Burnett A, Hall B, Parker KK: Agonistic properties of cannabidiol at 5-HT1a receptors. *Neurochem Res* 2005;30:1037-1043.

- 79 De Gregorio D, McLaughlin RJ, Posa L, Ochoa-Sanchez R, Enns J, Lopez-Canul M, Aboud M, Maione S, Comati S, Gobbi G: Cannabidiol modulates serotonergic transmission and reverses both allodynia and anxiety-like behavior in a model of neuropathic pain. *Pain* 2019;160:136-150.
- 80 Pandolfo P, Silveirinha V, dos Santos-Rodrigues A, Venance L, Ledent C, Takahashi RN, Cunha RA, Kofalvi A: Cannabinoids inhibit the synaptic uptake of adenosine and dopamine in the rat and mouse striatum. *Eur J Pharmacol* 2011;655:38-45.
- 81 Carrier EJ, Auchampach JA, Hillard CJ: Inhibition of an equilibrative nucleoside transporter by cannabidiol: a mechanism of cannabinoid immunosuppression. *Proc Natl Acad Sci U S A* 2006;103:7895-7900.
- 82 Laprairie R, Bagher A, Kelly M, Denovan-Wright E: Cannabidiol is a negative allosteric modulator of the cannabinoid CB1 receptor. *Br J Pharmacol* 2015;172:4790-4805.
- 83 Straiker AJ, Maguire G, Mackie K, Lindsey J: Localization of cannabinoid CB1 receptors in the human anterior eye and retina. *Invest Ophthalmol Vis Sci* 1999;40:2442-2448.
- 84 Stamer WD, Golightly SF, Hosohata Y, Ryan EP, Porter AC, Varga E, Noecker RJ, Felder CC, Yamamura HI: Cannabinoid CB(1) receptor expression, activation and detection of endogenous ligand in trabecular meshwork and ciliary process tissues. *Eur J Pharmacol* 2001;431:277-286.
- 85 Kohno M, Hasegawa H, Inoue A, Muraoka M, Miyazaki T, Oka K, Yasukawa M: Identification of N-arachidonylglycine as the endogenous ligand for orphan G-protein-coupled receptor GPR18. *Biochem Biophys Res Commun* 2006;347:827-832.
- 86 McHugh D, Page J, Dunn E, Bradshaw HB: Delta(9)-Tetrahydrocannabinol and N-arachidonyl glycine are full agonists at GPR18 receptors and induce migration in human endometrial HEC-1B cells. *Br J Pharmacol* 2012;165:2414-2424.
- 87 Console-Bram L, Brailoiu E, Brailoiu GC, Sharir H, Abood ME: Activation of GPR18 by cannabinoid compounds: a tale of biased agonism. *Br J Pharmacol* 2014;171:3908-3917.
- 88 Caldwell MD, Hu SS, Viswanathan S, Bradshaw H, Kelly ME, Straiker A: A GPR18-based signalling system regulates IOP in murine eye. *Br J Pharmacol* 2013;169:834-843.
- 89 MacIntyre J, Dong A, Straiker A, Zhu J, Howlett SE, Bagher A, Denovan-Wright E, Yu DY, Kelly ME: Cannabinoid and lipid-mediated vasorelaxation in retinal microvasculature. *Eur J Pharmacol* 2014;735:105-114.
- 90 Alhouayek M, Masquelier J, Muccioli GG: Lysophosphatidylinositols, from Cell Membrane Constituents to GPR55 Ligands. *Trends Pharmacol Sci* 2018;39:586-604.
- 91 Morales P, Jagerovic N: Advances Towards The Discovery of GPR55 Ligands. *Curr Med Chem* 2016;23:2087-2100.
- 92 Cherif H, Argaw A, Cecyre B, Bouchard A, Gagnon J, Javadi P, Desgent S, Mackie K, Bouchard JF: Role of GPR55 during Axon Growth and Target Innervation. *eNeuro* 2015; DOI: 10.1523/ENEURO.0011-15.2015.
- 93 Montell C: The TRP superfamily of cation channels. *Sci STKE* 2005;2005:re3.
- 94 Muller C, Morales P, Reggio PH: Cannabinoid Ligands Targeting TRP Channels. *Front Mol Neurosci* 2018;11:487.
- 95 De Petrocellis L, Orlando P, Moriello AS, Aviello G, Stott C, Izzo AA, Di Marzo V: Cannabinoid actions at TRPV channels: effects on TRPV3 and TRPV4 and their potential relevance to gastrointestinal inflammation. *Acta Physiol (Oxf)* 2012;204:255-266.
- 96 Hung CY, Tan CH: TRP Channels in Nociception and Pathological Pain. *Adv Exp Med Biol* 2018;1099:13-27.
- 97 Levine JD, Alessandri-Haber N: TRP channels: targets for the relief of pain. *Biochim Biophys Acta* 2007;1772:989-1003.
- 98 Barro-Soria R, Stindl J, Muller C, Foeckler R, Todorov V, Castrop H, Strauss O: Angiotensin-2-mediated Ca²⁺ signaling in the retinal pigment epithelium: role of angiotensin-receptor-associated-protein and TRPV2 channel. *PLoS One* 2012;7:e49624.
- 99 Reichhart N, Keckeis S, Fried F, Fels G, Strauss O: Regulation of surface expression of TRPV2 channels in the retinal pigment epithelium. *Graefes Arch Clin Exp Ophthalmol* 2015;253:865-874.
- 100 Gottschling S, Ayonrinde O, Bhaskar A, Blockman M, D'Agnone O, Schechter D, Suarez Rodriguez LD, Yafai S, Cyr C: Safety Considerations in Cannabinoid-Based Medicine. *Int J Gen Med* 2020;13:1317-1333.
- 101 Britch SC, Babalonis S, Walsh SL: Cannabidiol: pharmacology and therapeutic targets. *Psychopharmacology (Berl)* 2020;238:9-28.

- 102 Chesney E, Oliver D, Green A, Sovi S, Wilson J, Englund A, Freeman TP, McGuire P: Adverse effects of cannabidiol: a systematic review and meta-analysis of randomized clinical trials. *Neuropsychopharmacology* 2020;45:1799-1806.
- 103 Souza JG, Dias K, Pereira TA, Bernardi DS, Lopez RF: Topical delivery of ocular therapeutics: carrier systems and physical methods. *J Pharm Pharmacol* 2014;66:507-530.
- 104 Yellepeddi VK, Palakurthi S: Recent Advances in Topical Ocular Drug Delivery. *J Ocul Pharmacol Ther* 2016;32:67-82.
- 105 Tomida I, Pertwee RG, Azuara-Blanco A: Cannabinoids and glaucoma. *Br J Ophthalmol* 2004;88:708-713.
- 106 Richter A, Anton SF, Koch P, Dennett SL: The impact of reducing dose frequency on health outcomes. *Clin Ther* 2003;25:2307-2335; discussion 2306.