Seeing over the horizon – targeting the endocannabinoid system for the treatment of ocular disease

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The observation that marijuana reduces intraocular pressure was made by Hepler and Frank in the 1970s. Since then, there has been a significant body of work investigating cannabinoids for their potential use as therapeutics. To date, no endocannabinoid system (ECS)-modulating drug has been approved for clinical use in the eye; however, recent advances in our understanding of the ECS, as well as new pharmacological tools, has renewed interest in the development of ocular ECS-based therapeutics. This review summarizes the current state-of-affairs for the use of ECS-modulating drugs for the treatment of glaucoma and ocular inflammatory and ischemic disease.

Keywords: age-related macular degeneration; cannabinoid receptor 1; cannabinoid receptor 2; cannabinoids; diabetic retinopathy; endocannabinoids; glaucoma; inflammation; ocular; retina; uveitis.

Introduction

Cannabinoids can produce a variety of ocular effects, and prominent among these is a reduction in intraocular pressure (IOP). This latter effect has been the subject of extensive research, primarily with the purpose of developing effective therapeutics for glaucoma, a degenerating eye disease for which ocular hypertension (OH) is a prominent feature [1–5]. Research has now identified that the ocular actions of cannabinoids are mediated by an endogenous endocannabinoid system (ECS) (reviewed in Ref. [3]). Furthermore, in addition to glaucoma, alterations of the ECS have been reported in ocular inflammatory pathologies, including diabetic retinopathy (DR) and age-related macular degeneration (AMD) [6]. This suggests that ECS manipulation may be a promising target for treatment; however, no ECS-modulating drug has yet been approved for clinical use in the eye. Recent advances in our understanding of the ocular ECS, as well as new pharmacological tools, may rectify this situation. This review will discuss recent research that highlights potential new ECS targets for the treatment of ocular disease.

The ocular endocannabinoid system

The ocular ECS includes enzymes responsible for the production and degradation of endocannabinoids, as well as various receptors that endocannabinoid ligands target, including cannabinoid receptor (CB) 1 and CB2 [3, 7–34]. Additionally, endocannabinoids bind to a variety of “non-classical” cannabinoid receptors, including G-protein-coupled receptor (GPR) 18, GPR55, transient receptor potential vanilloid 1 (TRPV1), and peroxisome proliferator-activated receptors (PPARs) α, β/δ, and γ (reviewed in Ref. [35]). The reported presence of components of the ECS varies by ocular tissue type (see Tables 1–3). For example, the retina, the neuronal tissue responsible for the generation of vision, highly expresses various endocannabinoid-binding receptors and enzymes, whereas other tissues like the lens, the crystalline structure responsible for the focusing of light on the retina, is apparently devoid of ECS components [3, 7, 24].

2-Arachidonoylglycerol (2-AG) and anandamide (N-arachidonoyl ethanolamine; AEA) are two of the most...
well-studied endocannabinoids. Both 2-AG and AEA are found throughout ocular tissues, and like elsewhere in the central nervous system (CNS), 2-AG is present in higher concentrations than AEA [6, 36, 37]. Additionally, N-palmitylethanolamide (PEA), an analogue of AEA, has also been found in ocular tissues (see Table 1) [6, 17]. Endocannabinoid levels are maintained by the balance between on-demand synthesis and degradation. Endocannabinoid biosynthetic and degradative enzymes including diacylglycerol lipase (DGL) \(\alpha/\beta\), N-arachidonoyl phosphatidylethanolamine phospholipase D (NAPE-PLD), fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGL), and \(\alpha/\beta\)-hydrolase domain 6 (ABHD6) have all been localized to the mammalian eye ([12, 24, 26]; see Table 2 for a summary of expression).

Expression of CB1 is ubiquitous throughout the eye [8, 10–12, 14, 15]; however, both the presence and location of CB2 expression in a non-pathological state is controversial. Some pharmacological evidence has suggested that CB2 is present on the trabecular meshwork and therefore may be involved in aqueous humor dynamics [18, 19]. Furthermore, while several groups have reported finding CB2 mRNA and positive immunoreactivity throughout the retina [13, 25, 33], others have found CB2 immunoreactivity restricted to Müller cells [30].

Interpretation of published CB2 studies are difficult in that at least some drugs that act at CB2 also display activity at other receptors. For example, the CB2 “selective” drug (2-methyl-1-propyl-1\(^H\)-indol-3-yl)-L-naphthalenylmethanone (JWH 015) was recently shown to bind to CB1 at relatively

### Table 1: Presence and localization of endocannabinoids in the mammalian eye.

<table>
<thead>
<tr>
<th>Retina</th>
<th>Iris</th>
<th>TM</th>
<th>SC</th>
<th>CB</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>AEA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Cow, human, pig, rat</td>
<td>Matsuda et al. [7]; Bisogno et al. [9]; Stamer et al. [15]; Chen et al. [17], but see Straiker et al. [10]</td>
</tr>
<tr>
<td>2-AG</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Cow, human, rat</td>
<td>Bisogno et al. [9]; Straiker et al. [10]; Chen et al. [17]</td>
</tr>
<tr>
<td>PEA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Cow, human, rat</td>
<td>Bisogno et al. [9]; Straiker et al. [10]; Chen et al. [17]</td>
</tr>
</tbody>
</table>

+, Endocannabinoid is present in tissue; TM, trabecular meshwork; SC, Schlemm’s canal; CB, ciliary body.

### Table 2: Presence and localization of endocannabinoid synthesizing and degrading enzymes in the mammalian eye.

<table>
<thead>
<tr>
<th>Retina</th>
<th>Iris</th>
<th>TM</th>
<th>SC</th>
<th>CB</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>DGL(\alpha/\beta)</td>
<td>+(^a)</td>
<td>Mouse, rat</td>
<td>Hu et al. [24]; Zabouri et al. [26]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NAPE-PLD</td>
<td>+</td>
<td>Mouse, rat</td>
<td>Zabouri et al. [26]; Cécyre et al. [33]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FAAH</td>
<td>+</td>
<td>Cow, monkey, mouse, rat</td>
<td>Bisogno et al. [9]; Yazulla [3]; Yazulla et al. [12]; Njie et al. [22]; Hu et al. [24]; Bouskila et al. [27]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MGL</td>
<td>+</td>
<td>Mouse, rat</td>
<td>Njie et al. [21]; Yazulla [3]; Hu et al. [24]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ABHD6</td>
<td>+</td>
<td>Rat</td>
<td>Hu et al. [24]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+, Protein expression (immunohistochemistry, Western blotting); TM, trabecular meshwork; SC, Schlemm’s canal; CB, ciliary body. \(^a\)DGL\(\beta\) was associated only with blood vessels in the retina [24]. \(^b\)Pharmacological evidence only.

### Table 3: Presence and localization of classical and non-classical cannabinoid-binding receptors in the mammalian eye.

<table>
<thead>
<tr>
<th>Retina</th>
<th>Iris</th>
<th>TM</th>
<th>SC</th>
<th>CB</th>
<th>Species</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CB1</td>
<td>+, ‡</td>
<td>+, ‡</td>
<td>+</td>
<td>+, ‡</td>
<td>+, ‡</td>
<td>Guinea pig, human, mouse, monkey, pig, rat</td>
</tr>
<tr>
<td>CB2</td>
<td>+</td>
<td>‡</td>
<td>+</td>
<td>‡</td>
<td>Monkey, pig</td>
<td>Lu et al. [13]; Zhong et al. [18]; He et al. [19]; Lopez et al. [25]; Cécyre et al. [33], but see Porcella et al. [8], and Bouskila et al. [30]</td>
</tr>
<tr>
<td>GPR18</td>
<td>+, ‡</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>Mouse, rat</td>
<td>Caldwell et al. [32]; MacIntyre et al. [34]</td>
</tr>
<tr>
<td>GPR55</td>
<td>+(^b)</td>
<td>Monkey, pig</td>
<td>Kumar et al. [28]; Bouskila et al. [31]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TRPV1</td>
<td>+, ‡</td>
<td>Cat, monkey, rat</td>
<td>Yazzula and Studholme [16]; Nucci et al. [20]; Sappington et al. [23]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PPAR(\alpha)</td>
<td>‡</td>
<td>Cow, pig</td>
<td>Kumar et al. [28]; ‡Romano and Lograno [29]</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

+, Protein expression (immunohistochemistry, Western blotting); ‡, mRNA expression (RT-PCR). TM, Trabecular meshwork; SC, Schlemm’s canal; CB, ciliary body. \(^a\)Pharmacological evidence only. \(^b\)Staining exclusive to rods. \(^c\)Pharmacological data from ophthalmic artery only.
Intraocular pressure

Components of the ECS are highly expressed on tissues responsible for IOP regulation [2, 4, 39]. IOP is the product of the difference between aqueous humor production and outflow. Aqueous humor is a clear liquid that serves as a circulatory system within the eye, nourishing and removing wastes from avascular areas of the eye, and helps maintain eye shape, which is important for proper optics. Aqueous humor is produced by the bilayered ciliary epithelium, and once formed, flows into the posterior chamber before circulating to the anterior chamber (reviewed in Ref. [46]). Outflow is complex and occurs through either the conventional pathway, which flows from the anterior chamber through the trabecular meshwork and into Schlemm’s canal, or through the uveoscleral pathway, which involves the flow of aqueous humor from the iridocorneal angle through to the ciliary body before draining into the supraciliary and suprachoroidal spaces [47]. The amount of flow through either the uveoscleral pathway or the classical pathway is dynamic and varies significantly between species. As such, the development of new IOP-modulating drugs may be confounded by species differences; an effective ocular hypotensive in one species, such as rabbits, may not necessarily translate into significant changes in IOP in humans [47].

The effects of the phytocannabinoid Δ⁹-tetrahydrocannabinol (Δ⁹-THC) and the synthetic agonist (R)-(−)-[2,3-Dihydro-5-methyl-3-(4-morpholinylmethyl) pyrrolo[1,2,3-de]-1,4-benzoxazin-6-yl]-1-naphthalenylmethanone mesylate (WIN 55,212-2; WIN) on IOP have been well studied [48–52]. These cannabinoids appear to lower IOP by a CB1-mediated mechanism that is β-adrenergic receptor dependent [53, 54], similar to what was found elsewhere [55]. So far, there has been a limited number of human studies with variable results using Δ⁹-THC and WIN in clinical trials [52, 56]; however, new therapeutic strategies and targets, combined with localized drug delivery, may provide better methods to increase the efficacy and duration of IOP-lowering actions, while reducing systemic and CNS side effects.

Cannabinoids, intraocular pressure, and glaucoma

Hepler and Frank [41] first described the IOP-lowering effects of smoked marijuana in 1971. Since then, ECS-modulating drugs have been extensively investigated as potential therapeutics for the treatment of OH [1, 2, 4, 39], a risk factor for glaucoma [42]. Glaucoma, the second leading cause of blindness worldwide, is a progressive neurodegenerative disorder characterized by retinal ganglion cell (RGC) loss [5]. However, the mechanisms leading to RGC death in glaucoma are not yet fully understood; IOP is the only modifiable risk factor and therefore is currently the only target for therapy [43–45]. Success of cannabinoids for the treatment of OH in clinical trials has been limited, primarily due to their variable efficacy, most likely a result of their short duration of action in reducing of IOP, potential receptor desensitization, and behavioral side effects [4]. The following sections will summarize the current state-of-affairs for the use of ECS-modulating drugs for control of IOP as well as recent evidence supporting cannabinoid-mediated (and IOP-independent) RGC neuroprotection in glaucoma.

Neuroprotection

There is currently no approved glaucoma therapeutic that directly targets RGC loss. Such a drug would be beneficial, as many patients with glaucoma continue to have progressive visual field loss despite IOP control [45]. Furthermore, patients where IOP never becomes elevated but have
glaucoma (so-called normal tension glaucoma) may also benefit from a neuroprotective-targeted therapy [45].

Triggers that ultimately lead to RGC death in glaucoma are likely multifactorial, although consistent evidence suggests that this death ultimately occurs via caspase-dependent apoptosis [57]. Once caspases are activated, the retinal damage is irreversible [58], and therefore, potential neuroprotective targets must be upstream of caspase activation. Several hypotheses have been proposed as mechanisms leading to RGC loss. These include excitotoxicity, loss of neurotrophic support at the optic nerve, and oxidative stress, and it is likely that more than one of these factors may be contributing (as reviewed in Ref. [57]). Given the complexities of RGC death in glaucoma, the fact that ECS-modulating drugs can target multiple signaling pathways could be advantageous [59, 60]. The neuroprotective mechanisms of ECS-modulating drugs include decreasing glutamatergic signaling via presynaptic modulation of voltage-gated Ca\(^{2+}\) channels and K\(^{-}\) channels with a resultant reduction in neurotransmitter release [61–63], activation of pro-survival pathways, such as by activation of protein kinase B (Akt) and extracellular signal-regulated kinases (ERK) 1/2 [64–67], and reduction of immune cell (e.g. resident microglia) activation and migration, thereby decreasing nitric oxide production and release of pro-inflammatory cytokines [68–72].

Administration of cannabinoid drugs has produced RGC neuroprotection in a number of different models [73–76]. Notably, Pinar-Sueiró et al. [75] showed significant neuroprotection with topical administration of WIN in a model of transient high-IOP ischemia-reperfusion injury. The WIN-induced increase in RGC survival was blocked with co-administration of the CB1 antagonist N-(piperidin-1-yl)-5-(4-iodophenyl)-1-(2,4-dichlorophenyl)-4-methyl-1H-pyrazole-3-carboxamide (AM 251), suggesting a CB1-mediated reduction. However, as this model involves changes in aqueous humor dynamics and WIN-induced reductions in IOP were not measured, it is possible that this neuroprotective effect was in part IOP-mediated. Meanwhile, Slusar et al. [37] showed that systemic administration of cyclohexylcarbamic acid 3′-(aminocarbonyl)-[1,1′-biphenyl]-3-yl ester (URB 597), an inhibitor of the AEA degrading enzyme FAAH, was neuroprotective in a rat axotomy model, an IOP-independent model of RGC loss. This effect was inhibited by the CB1 antagonist AM 251, but not the CB2 antagonist 6-iodo-2-methyl-1-[2-(4-morpholinyl)ethyl]-1H-indol-3-yl(6-methoxyphenyl) methanone (AM 630), suggesting that this effect was also CB1-dependent [37]. Investigation of the neuroprotective effect of ECS-modulating drugs is still relatively new. With a better understanding of the mechanisms leading to RGC death, further development of ECS-modulating drugs may lead to more effective therapeutics for the treatment of glaucoma.

**New horizons in glaucoma?**

CB1 agonists that reduce IOP, such as WIN and Δ\(^{9}\)-THC, have been well studied as potential glaucoma therapeutics in both preclinical and clinical studies [48–52, 77]. However, to date, no cannabinoids drug has been approved for the treatment of glaucoma. This lack of success of cannabinoid therapeutics in glaucoma is largely attributed the short duration of action and possible behavioral and systemic side effects of cannabinoids, and the existence of alternative effective ocular hypotensive medications [43, 78, 79]. More recently, several new pharmacological strategies have been developed, which may reduce the limitations of CB1 agonists. Some of these strategies include the use of biased agonists or allosteric modulators and the use of degradative enzyme inhibitors [80–82]. In particular, modulating CB1 through allosteric modulation and/or inhibition of endocannabinoid-degrading enzymes (like FAAH and MAGL) may be able to increase duration of action, reduce desensitization, and limit psychotropic effects [80, 82–85].

The effects of administered endocannabinoids such as 2-AG and AEA are extremely short-lived and therefore not ideal as a therapeutic strategy [22, 53]. One method to increase the duration of effect would be to increase the amount of endocannabinoid available at the receptor through the use of inhibitors of endocannabinoid metabolism [82–84]. For example, Njie et al. [22] demonstrated that AEA administered in a porcine ocular perfusion model produced an increase in outflow facility, but this effect only persisted for 30 min. However, when AEA was co-administered with URB 597, a FAAH inhibitor, this effect was prolonged to 5 h [22]. Similarly, in another study using the same model, administration of the non-selective MGL/FAAH/ABHD6 inhibitor 5-[(1,1′-biphenyl)-4-yl]methyl]-N,N-dimethyl-1H-tetrazole-1-carboxamide (LY 2183240) increased outflow facility with a comparable duration of action to that seen with AEA+URB 597 [21]. Use of URB 597 was also found to be neuroprotective in an acute model of transient high-IOP retinal ischemia-reperfusion injury [20] and a model of IOP-independent RGC loss [37]. The neuroprotective effects of URB 597 were independent of concomitant exogenous AEA dosing [20, 37].

Although to the best of our knowledge there are no published studies on the use of selective inhibition of 2-AG metabolizing enzymes (such as MGL and ABHD6).
in a model of glaucoma, other studies have examined neuroprotective effects of inhibiting 2-AG degradation. These include a model of traumatic brain injury, where a significant reduction in lesion size was found with administration of the ABHD6 inhibitor N-methyl-N-[[3-(4-pyridinyl)phenyl]methyl]-4′-(aminocarbonyl) [1,1′-biphenyl]-4-yl carbamic acid ester (WWL 70) [86]. Additionally, in an experimental model of Parkinson disease, significant neuroprotection occurred after administration of the MGL inhibitor 4-[(cis,1,3-benzodioxol-5-yl) hydroxymethyl]-1-piperidinecarboxylic acid 4-nitrophe- nyl ester (JZL 184) [87, 88]. Taken together, these results suggest that use of enzyme-inhibiting drugs may be more appropriate for chronic treatment of neurodegeneration than administration of endocannabinoids alone.

An allosteric site on CB1 was recently characterized [89], and since then, a few allosteric-modulating drugs have been investigated [80, 85, 90–93]. Allosteric modulators bind to a site other than the natural, or orthosteric, binding site. Compounds binding to the allosteric site can affect the affinity and/or efficacy of the orthosteric ligand to the receptor (e.g. endocannabinoids to CB1) [94]. In doing so, allosteric modulators decrease the potential of CB1-mediated psychotropic effects by producing a more favorable therapeutic index (decreased dose) for the use of CB1 orthosteric ligands with reduced receptor desensitization (improved long-term efficacy) [80]. Furthermore, as upregulation of cannabinoid receptors has been reported in various disease states, including the eye [20], use of positive allosteric modulators (PAMs) may prove effective for increasing the localized actions of endocannabinoids at specific tissue sites [80]. So far, relatively few studies have been published using CB1 PAMs, and none yet so far in the eye. Pamplona et al. [85] studied the use of the endogenous CB1 PAM lipoxin A₄ in a model of β-amyloid-induced memory impairment. Mice receiving lipoxin A₄ performed significantly better in a spatial memory task compared with controls, possibly suggesting some neuroprotective effect. Although work in this area is still in the very early stages, the potential for these types of drugs in chronic disease is large and therefore is an important area of study.

Another new ECS-modulating drug strategy has been the use of ligand-directed, or biased, signaling [81, 93]. CB1 couples to three major Gα proteins (Gᵢ, Gₒ, and Gₛ) and can therefore elicit varied signaling pathways (reviewed in Ref. [95]). Studies analyzing downstream signaling of CB1 ligands have found that not all CB1 agonists activate the G proteins in the same manner [81, 96, 97]. Use of ligands that are biased for a particular pathway may be able to elicit favorable results, while minimizing side effects [98]. Typically, CB1 activates Gᵢ-mediated pathways; however, activation of CB1 by WIN has also been reported to activate Gₛ and Gₛ-mediated pathways (reviewed in Refs. [81, 95]). Additionally, CB1 has been shown to dimerize to a number of different receptors including A₂a adenosine receptor, β₂ adrenergic receptor, D₂ dopamine receptor, orexin 1 receptor, and μ, κ, and δ opioid receptors, which may result in distinct G-protein- or non-G-protein-biased signaling depending on the receptor interactions (reviewed in Refs. [81, 95]). Future work will determine which of these signaling pathways will provide the most therapeutic benefit and allow further development of biased ligands for specific ocular diseases.

Apart from CB1, studies of other ECS targets have been examined for the treatment of glaucoma. These include the non-CB1/CB2 receptors TRPV1, GPR18, and PPARα, all of which can bind cannabinoids [35]. TRPV1 is a cation-selective channel, expressed on both neuronal and microglial cells in the retina [16, 20, 23]. TRPV1 is activated by the endocannabinoid AEA, as well as vanillic acids, and is suggested to be important in cellular excitability (reviewed in Refs. [82, 99]). Investigation of the role of TRPV1 in pressure-induced RGC death has led to some opposing results, calling into question the exact role of TRPV1 in the retina [23, 99–103]. Depending on the study, researchers have concluded that TRPV1 activation can either be neuroprotective [103] or is a major contributor to pressure-induced damage in the eye [23, 100]. The expression of TRPV1 in models of ischemia/reperfusion and OH has been varied. However, recent work found that RGC damage appears to be exacerbated in TRPV1⁺ mice [103], suggesting that TRPV1 in the retina is neuroprotective. Mechanisms of TRPV1 activation to promote RGC survival are complex, but may involve promotion of RGC excitability during retinal stress, as well as release of neuroprotective cytokines, such as interleukin (II) 6, from glial cells (as reviewed in Refs. [99, 102]). Working investigating the role of TRPV1 in models of glaucoma is ongoing; however, its manipulation, either on its own or in conjunction with other cannabinoid receptors, holds therapeutic potential.

GPR18 is a recently deorphanized G-protein-coupled receptor for which N-archidonoylglycine (a metabolite of AEA) and abnormal cannabidiol (Abn-CBD) are ligands [104, 105]. GPR18 is expressed in the retina, ciliary epithelium, corneal epithelium, and trabecular meshwork [32, 34]. Application of GPR18 agonists reduced IOP independently of CB1, CB2, and GPR55 [32, 105]. Additionally, this IOP-lowering effect was independent of β- arsonin 1 and 2 [32], unlike the actions of CB1 [53]. This suggests that drugs modulating GPR18 may be a good target for IOP modulation by a distinct mechanism than CB1 and...
therefore devoid of psychotropic side effects. In the retina, GPR18 co-localizes with retinal microvasculature and application of its agonists causes vasorelaxation [34]; however, the effects of GPR18 in retinal function have yet to be studied.

PEA is an analogue of AEA; however, this endocannabinoid does not bind to either CB1 or CB2, but competes with AEA for degradation by FAAH. Although increases in endogenous PEA levels could potentially result in increases in AEA and AEA-mediated activation of CB1, when administered topically PEA does not lower IOP [106]. However, two small clinical trials have investigated the effect of oral PEA in glaucoma. One clinical trial involved oral administration of PEA to patients with primary open angle glaucoma and found a significant reduction in IOP compared with baseline measurements [107]. Another small clinical trial found similar results in patients with normal tension glaucoma: IOP was reduced and visual fields parameters improved when measured at a 6-month follow-up [108]. Some effects of PEA have been attributed to actions at non-CB1/CB2 receptors. A study by Kumar et al. [28] found that PEA increased ocular outflow facility in a perfused porcine model, an effect partially attributed to PPARα activation. Interestingly, the authors of the article [28] also concluded that this PEA-induced increase in outflow also involves GRP55, as shRNA knockdown of GRP55 partially attenuated the effect [28]. In contrast, an IOP-lowering effect remained intact in GPR55 knockout mice when administered the non-selective GPR55 agonist Abn-CBD, suggesting that this receptor is not involved in IOP regulation [32]. Additionally, activation of PPARs by PEA (and AEA) caused vasorelaxation of bovine ophthalmic artery and was blocked by inhibitors of nitric oxide synthase and large conductance Ca\(^{2+}\)-activated K\(^{+}\) channels [29]. Further, PEA administration after traumatic CNS injury is neuroprotective, potentially by decreasing edema and modulation of inflammation (as reviewed in Ref. [109]).

Taken together, this evidence suggests that PEA may be effective at lowering IOP by a mechanism independent of CB1 and CB2 and that given the neuroprotective actions of PEA, future studies to analyze the effect of PEA on RGCs are warranted.

The use of ECS-modulating drugs for the treatment of glaucoma remains an as yet unrealized area. As demonstrated by the recent research, there is significant new evidence that warrants further study into novel ECS-based therapeutics, targeting both IOP and RGC loss. Given that there is currently no treatment that directly targets the RGC loss in glaucoma, the potential of ECS-based therapeutics for ocular neurodegenerative disease merits further exploration in both appropriate experimental models and in clinical studies.

The endocannabinoid system and ocular inflammation

Cannabinoid researchers have been actively investigating the role of the ECS in the immune response since 1993 [110]; however, until very recently, its role in the ocular immune response has not been examined. The ECS is a potential therapeutic target for immunomodulation with a growing body of evidence indicating that both CB1 and CB2 signaling may contribute (reviewed in Refs. [60, 111, 112]). Activation of CB2, specifically, is anti-inflammatory in a number of tissues and organs, including the eye [113–115]. CB2 was cloned from a human promyelocytic leukaemia cell line HL60 and rat macrophages and was referred to as the peripheral cannabinoid receptor [110, 116]. CB2 has since been localized to all examined subtypes of leukocytes [117–121], in addition to other non-immune cells, including endothelial cells [122], osteoclasts [55], with reports in neurons and peripheral nerve terminals [60]. Within the subsets of immune cells, CB2 mRNA levels vary being highest in B-cells > natural killer cells > monocytes > polymorphonuclear cells > T8 cells > T4 cells [117]. CB2 is also expressed by antigen-presenting cells (APCs), including macrophages, dendritic cells, and microglia [123, 124]. CB2 immunomodulation has been extensively reviewed by others [120, 125, 126]. Briefly, it has been shown that CB2 agonists can decrease release of inflammatory mediators including cytokines, chemokines, and adhesion molecules, inhibit chemotaxis of immune cells, and modulate proliferation and antigen presentation [118, 119, 121, 124, 127]. Furthermore, during inflammation, CB2 expression is increased in vivo and in vitro, and this may result in alternations in CB2 signaling [128, 129].

The ocular immune system

The eye is a unique organ of the body, encompassing both the peripheral nervous system (PNS) and CNS immune system. Immune cells associated with the peripheral region of the eye, including the cornea, ciliary body, iris, choroid, include populations of cells such as macrophages and dendritic cells [130–132]. The retina is an extension of the CNS and thus includes resident immune cells such as microglia [133]. The complexity of the ocular immune system is increased by the immune privilege status of the
eyelid, which is achieved by a physical blood-ocular barrier (formed by the blood-aqueous barrier and the blood-retinal barrier [BRB]), absence of lymphatic pathways, immunomodulatory factors released in the aqueous humor, immunoregulation via cell-to-cell contact mechanisms with corneal endothelium and iris-pigmented epithelium, and APC development of antigen tolerance [133-137]. Together, these adaptations help to maintain the ocular microenvironment and proper ocular function.

Following trauma or inflammation in the eye, the blood-ocular barriers are compromised, and resident APCs, including macrophages, dendritic cells, and microglia, activate and recruit other immune cells of innate and adaptive immune systems. This recruitment is facilitated by cytokines and chemokines. In the case of ocular innate immune response, neutrophils, basophils, eosinophils migrate from systemic circulation to the site of inflammation [137]. The adaptive immune response is based on APCs processing and displaying antigens, which are detected and recognized by T-cells to elicit their activation [138]. Both systems are intricately linked together and constitute the cells that make up the ocular immune system.

Cannabinoids and uveitis

The immune response within the eye can become exacerbated during inflammatory disease states such as uveitis and override the immune privilege compensatory mechanism, threatening eyesight. Uveitis is a broad term that describes inflammation, arising from either infectious or non-infectious origins, occurring within specific regions of the eye including the uvea, which comprises the iris, ciliary body, and choroid. Additionally, inflammation within the sclera, retina, vitreous, and optic nerve can also be classified as uveitis. The location of the inflammation within different anatomical regions allows several designations for the type of uveitis: anterior, intermediate, and posterior or throughout the eye (pan-uveitis [139]).

Current pharmacological treatments for uveitis include classical immunosuppressive drugs, such as corticosteroids, as well as newer biological agents. However, chronic corticosteroid use can lead to numerous adverse effects, including cataracts and glaucoma, and biologicals are extremely costly [140, 141].

Several recent studies have indicated the potential of the ECS, and in particular CB2, as a therapeutic target to treat uveitis and other ocular inflammatory and fibrotic diseases [113, 114]. The anti-inflammatory effects of selective CB2 agonist, (6aR,10aR)-3-(1,1-dimethylbutyl)-6a,7,10a-tetrahydro-6,6,9-trimethyl-6H-dibenzo[b,d]pyran (JWH 133), have been examined in experimental uveitis [113]. This study used a pan-uveitis model based on a CD4+ T-cell-mediated response to interphotoreceptor binding protein peptide, with a primary focus on pathological changes in the retina. JWH 133 dose-dependently improved both clinical and histopathological scores with respect to inflammatory cell infiltration and retinal structural changes. This was accompanied by blunted cytokine production, including interferon (IFN) γ, tumor necrosis factor (TNF) α, IL-6, and IL-10, and completely ablated monocyte chemoattractant protein 1 (CCL2) production in JWH 133-treated animals. Xu et al. [113] attributed these anti-inflammatory effects to the direct actions of JWH 133 on lymphocytes.

More recently, Toguri et al. [114] further demonstrated the potential of CB2 agonists as anti-inflammatory agents during uveitis. This study used real-time non-invasive intravital microscopy to measure leukocyte-endothelial adhesion in the iridial microcirculation in a model of endotoxin-induced uveitis by intraocular injection of lipopolysaccharide (LPS). Topical application of the cannabinoid 4-[4-(1,1-Dimethylheptyl)-2,6-dimethoxyphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-methanol (HU 308), a CB2 agonist, significantly decreased leukocyte-endothelial adhesion, and this effect was blocked by the CB2 antagonist AM 630. Toguri et al. [114] reported that CB2 activation reduced transcription factors nuclear factor κB and activator protein 1, accompanied by decreased cytokines (including TNF-α, IL-1β, IL-6, IL-10, IFN-γ) and chemokines (CCL5 and CXCL2). These effects were antagonized by AM 630. Notably, in this study, CB2-activating drugs performed better than three clinically used anti-inflammatory drugs to treat uveitis, e.g. dexamethasone, prednisolone, nafinac.

Relatively few studies to date have specifically examined the actions of endocannabinoids in uveitis. In contrast to other studies that reported the anti-inflammatory actions of cannabinoids in experimental uveitis, Altinsoy et al. [142] reported that introduction of AEA via intracellular injection in a rabbit model of LPS-induced uveitis exacerbated all measured outcomes of inflammation. The increase in inflammation seen with AEA was reduced by both CB1 antagonism. In contrast to Altinsoy et al. [142], Toguri et al. [115] recently reported that the cannabinoid WIN, given via the intravenous route, reduces leukocyte recruitment in experimental uveitis generated by systemic LPS injection in rats, and this beneficial effect of WIN was reduced by both CB2 and CB1 antagonism. In support of this latter study, a reduction in retinal damage was also reported following treatment with either WIN or Δ^2-THC.
Cannabinoids, diabetic retinopathy, and age-related macular degeneration

DR is a retinal disease associated with chronic inflammation and neovascularization (reviewed in Ref. [144]). Breakdown of the BRB and retinal cell death are clinical features of DR, and are thought to occur as a result of hyperglycemia-induced oxidative stress leading to increased release of proinflammatory cytokines (reviewed in Ref. [145]). A number of studies have examined the involvement of the ECS and cannabinoids in experimental rodent DR models. The effects of the cannabidiol (CBD) were examined in DR induced in rats by injection of streptozotocin [146]. Chronic CBD treatment was anti-inflammatory and resulted in a significant decrease of BRB breakdown, oxidative stress, and vascular endothelial growth factor expression. This study also reported a reduction in activated p38 mitogen-activated protein kinase (MAPK), a stress-activated protein kinase that is a downstream target of proinflammatory cytokines and oxidative stress and a reduction in retinal cell death [146]. The receptor target for the actions of CBD in the retina was not extensively explored in this study; however, other mechanistic studies have reported pleiotropic actions of CBD including antagonism of CB1, as well as agonist actions at adenosine A<sub>3</sub> receptors, 5-hydroxytryptamine 1A receptors, and nuclear PPAR<sub>γ</sub> receptors (reviewed in Ref. [147]). In keeping with a potential CB1 antagonistic role of CBD, a more recent study by El-Remessy et al. [148] reported that genetic deletion or chronic receptor blockade of CB1 in a mouse model of DR prevented retinal cell death. Blocking CB1 receptor signaling in these models was associated with reduced oxidative stress, Müller cell activation, as well as decreased levels of proinflammatory cytokines and adhesion factors (intercellular adhesion molecule 1 and vascular cell adhesion molecule 1) and reduced activation of stress signaling pathways p38 and Jun N-terminal kinase MAPKs.

Although endocannabinoid and cannabinoid receptor protein levels were not reported in these rodent DR models, Matias et al. [6] examined endocannabinoid levels in post-mortem eyes from patients with DR. The authors reported elevated AEA levels in the retina, which they attributed to dysfunctional ECS signaling. In the same study, the authors also found elevated AEA levels in retinas of post-mortem eyes of patients with AMD. AMD is a degenerative retinal disease leading to retinal pigmented epithelium (RPE) atrophy, in which oxidative stress is a contributing factor [149]. Exposing primary human RPE cells and an RPE cell line to hydrogen peroxide-induced oxidative stress upregulated expression of CB1 and CB2 receptors and downregulated FAAH expression [149]. This latter finding would imply that decreases in FAAH could contribute to the reported increased levels of AEA in post-mortem AMD eyes.

Although there is a lack of information on the effects of cannabinoids in in vivo preclinical models of AMD, Wei et al. [149] reported expression of CB1 and CB2 receptors as well as FAAH in human RPE cells. Treatment of RPE cells with cannabinoids, including the CB1 and CB2 agonist CP 55,940 and the CB2 agonist JWH 015, protected cells from oxidative stress-induced cell death by reducing ROS and activating of P13K/Akt pro-survival signaling pathways. In a follow-up study, Wei et al. [150] investigated the effects of the selective CB1 receptor antagonist SR 141716 and receptor inhibition by siRNA in primary human RPE cells exposed to hydrogen peroxide-induced oxidative stress. Their findings suggest that attenuating CB1 receptor signaling ameliorates oxidative stress-induced cell death and enhances activation of P13K/Akt [150]. These studies support the hypothesis that cannabinoid signaling plays a role in AMD. However, further studies in retinal ischemic pathologies such as DR and AMD are clearly required to resolve changes in ECS signaling in these pathologies as well as to determine the most appropriate therapeutic approach.

Final thoughts

Through the development of better pharmacological tools and an increased understanding of the ocular ECS, there is significant potential for the development of new ECS-targeted therapies for ocular diseases. With regard to glaucoma, drugs targeting cannabinoid receptors, such as CB1, may be advantageous as adjuncts to existing clinical hypotensive agents due to their dual actions in lowering IOP and providing neuroprotection of RGCs. Furthermore, development of CB1 allosteric modulators and enzyme inhibitors for ocular use would maintain the benefits of CB1 activation, and may result in a more favorable therapeutic index [80, 82–85].

The ECS is important in the modulation of the ocular immune response [113, 114]. Studies of CB1 in inflammation...
have variable results; however, in the treatment of ocular inflammation, there is convincing evidence that suggests that CB2 may be a clinically relevant target [114, 142]. So far, most research has been focused on modulating specific components of the ocular inflammatory response. However, given that the eye has both elements of the PNS and CNS immune responses, as well as both innate and adaptive immune systems, additional studies encompassing these complexities are still required before a CB2 agonist or other ECS modulators can reach the clinic [130–133]. Additional studies examining ECS modulation in ocular tissues in which both receptors and endocannabinoids have been identified, e.g. the cornea, may provide evidence for additional analgesic and anti-inflammatory therapeutic indications [6, 11, 151–153].

A significant amount of work in the last few years has granted new insights into the function of the ocular ECS, particularly in ocular disease. However, not all observed effects of ECS-modulating drugs can currently be fully explained, and it appears that in some cases we may be missing parts of the puzzle. Further studies encompassing the complexities of both the ECS and ocular disease pathologies will enable a better understanding of ECS-modulating drug actions and may enable the generation of better targeted and effective therapeutics. Given the current momentum of discoveries in this area, it is quite possible that ECS-modulating drugs could soon be clinically available for the treatment of ocular disease.

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References


19. He F, Song ZH. Molecular and cellular changes induced by the activation of CB2 cannabinoid receptors in trabecular meshwork cells. Mol Vis 2007;13:1348–56.


84. Pertwee RG. The therapeutic potential of drugs that target cannabinoid receptors or modulate the tissue levels or actions of endocannabinoids. AAPS J 2005;7:E625–54.


