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Cannabidiol As a Putative Novel Therapy for Diabetic Retinopathy: A Postulated Mechanism of Action as an Entry Point for Biomarker-Guided Clinical Development

GI Liou[1,*], **AB El-Remessy**[1,3,4], **AS Ibrahim**[1,5], **RB Caldwell**[1,2,4], **YM Khalifa**[1,5], **A Gunes**[6], and **JJ Nussbaum**[1]

[1] Department of Ophthalmology, Medical College of Georgia, Augusta, GA, USA

[2] Vascular Biology Center, Medical College of Georgia, Augusta, GA, USA

[3] Program in Clinical and Experimental Therapeutics, University of Georgia

[4] VA Medical Center, Augusta, GA, USA

[5] Department of Biochemistry, Faculty of Pharmacy, Mansoura University, Mansoura, Egypt

[6] Department of Medical Sciences, Clinical Pharmacology, Uppsala University, Uppsala, Sweden

Abstract

Diabetic retinopathy is a leading cause of blindness in the Western world. However, treatment options for diabetic retinopathy are limited and display poor efficacy with marked patient-to-patient variation in therapeutic outcomes. Discovery of new molecular entities acting on mechanistically novel biological pathways remains as one of the key research priorities in diabetic retinopathy. Moreover, given the variable success of the existing treatment modalities, a targeted and personalized drug development strategy could be more fruitful for rational and successful transition of preclinical discoveries to the clinical realm. This review is focused on cannabidiol, a non-psychoactive native cannabinoid, as an emerging and novel therapeutic modality based on systematic studies in animal models of inflammatory retinal diseases including diabetic retinopathy - one of the retinal diseases associated with vascular neuroinflammation. We present the postulated and preclinically documented novel mechanisms that may underlie cannabidiol mode of action in diabetic retinopathy. We discuss the interindividual variation in pharmacokinetic pathways as well as in the *SLC29A1* gene, a molecular target for cannabidiol. We emphasize that the novel mode of action of cannabidiol and the previous failures with nontargeted interventions in diabetic retinopathy collectively demand a more rational and personalized clinical development strategy for compounds that have shown promise at the preclinical stage. Moreover, it is noteworthy that ophthalmology, as a medical specialty, has fewer examples (e.g., compared to oncology) of personalized medicine and biomarker applications thus far. Understanding the biological action of cannabidiol in preclinical studies is therefore a rational first step to proactively map the pertinent biomarker strategies in clinical proof of concept studies in diabetic retinopathy, and to allow advances at the hitherto neglected intersection of personalized medicine and ophthalmology.

[*]Corresponding author: Dr. Gregory I. Liou, Department of Ophthalmology, Medical College of Georgia, 1120 15th Street, Augusta, GA 30912; Tel: (706) 721-4599; Fax: (706) 721-1158; giliou@mcg.edu.

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Keywords

Anti-inflammation; Cannabidiol; Diabetic retinopathy; drug mode of action and biomarkers; targeted intervention

1. Introduction

Diabetic retinopathy (DR) is a retinal disease associated with vascular neuroinflammation and one of the leading causes of blindness in the Western world, affecting approximately three-fourths of diabetic patients within 15 years after the onset of the disease [1,2]. Even though lack of glycemic control increases the risk for the microvascular complications of diabetes, the prevalence of and progression to diabetic retinopathy show substantial person-to-person and population variation despite clinical interventions to achieve strict glycemic control. The recommended treatment for DR is laser photo-coagulation but the procedure also destroys the neural tissues [3,4]. Therefore, there is a great clinical need and growing research interest for the development of new non-invasive therapies for DR based on novel drug targets.

One recent strategy in DR research has focused on delineating the inflammatory and neurodegenerative processes involved. To this end, we identified new non-invasive and receptor-based therapies for mitigating the retinal damage associated with diabetes. These include cannabidiol (CBD) that activates, in preclinical models, a self-defense system against inflammation and neurodegeneration. On the other hand, drug candidates that act on novel molecular mechanisms demand commitment to a rational research program for successful transition from preclinical models to clinical proof of concept studies, and planning for the attendant development of biomarkers of individual variability in drug response and toxicity. These efforts can be collectively facilitated by a sound knowledge of the biological pathways and molecular targets impacted by a new compound or drug candidate. Hence, the aim of this paper is to present a preclinical mechanistic analyses of the anti-inflammatory pharmacodynamic activity associated with CBD and when available, present evidence on genetic variation in the pathways related to the metabolism, pharmacokinetics and the postulated mode of action of CBD in treatment of DR.

2. Diabetic retinopathy, public health significance and the need for new diagnostics

DR is a well-defined chronic ocular disorder that can lead to the loss of sight. Over 20 million adults (or 9.6% of the total population) in the United States currently have diabetes. Of this group, over 12,000 patients will be diagnosed with new-onset blindness annually, making it the leading cause of legal blindness in Americans 20–74 years of age [5]. An estimated $132 billion was spent on the direct healthcare and indirect consequences of diabetes in 2002, based on the analysis by the American Diabetes Association. Regular ophthalmic screening of diabetic patients is more cost-effective than other routinely provided health exams, making it an important health investment for society [6,7]. Moreover, due to the variable success of the existing treatment modalities, a personalized drug development strategy could be more fruitful for rational and successful transition of preclinical discoveries to the clinical realm. In assessing and managing diabetes, and specifically DR, a holistic approach is thus recommended: improved preventive care, earlier diagnosis, more intensive disease management, and the use of new medical diagnostics, including biomarkers for prediction of susceptibility to DR as well as response to the available treatments, to minimize the trial and error approach in finding the optimal therapeutic modality. These measures, if available and applied systematically, can meaningfully improve patients' quality of life, and reduce the health expenditures for related services. As it will be noted in the subsequent sections of this paper, there is however a shortage

of new diagnostics in ophthalmology that can be coupled with existing or new treatments for DR; this new class of diagnostics are also known as theragnostic tests which are intended to personalize therapeutic interventions with the overarching goal of improving their efficacy and safety.

2.1. Clinical characterization of diabetic retinopathy

Retinopathy is common in diabetics and occurs, to some and highly variable degree, in almost all patients with the disease for 20 years or more, even though the type I diabetics have a higher incidence of retinopathy [1]. Loss of retinal pericytes and alterations in retinal blood flow are preclinical changes that are not often detectable during routine clinical practice [8,9]. The earliest detectable type of DR is nonproliferative diabetic retinopathy (NPDR), which is further clinically subdivided into mild, moderate and severe categories. Retinal venous dilation and microaneurysms are the first alterations detectable by ophthalmoscopy. Subsequently, intraretinal hemorrhage and exudation may occur. Intraretinal leakage leads to macular edema, which if untreated may lead to irreversible vision loss and legal blindness. As hyperglycemia persists, the disease progresses to moderate and severe forms of retinopathy. Severe NPDR presents with hemorrhages in all quadrants and venous beading suggesting dilated capillaries as indicated by decreased retinal circulation and intraretinal microvascular abnormalities (IRMA) [10]. In short, advanced NPDR is characterized by increased ischemia, resulting in more severe vascular permeability, widespread hemorrhaging, venous abnormalities, and IRMAs. All stages of the disease prior to new retinal vessel development are referred to as NPDR.

Once proliferation of new blood vessels begins, the disease progresses to proliferative diabetic retinopathy (PDR). This stage is characterized by the onset of ischemia-induced new vessel proliferation from the optic nerve head or elsewhere in the retina. These new vessels are extremely fragile and tend to bleed easily, resulting in vitreous hemorrhage. Over time the neovascularization tends to undergo fibrosis and contraction, leading to complex traction retinal detachments. In addition, new vessels can sprout on the iris and in the trabecular meshwork of the anterior chamber resulting in neovascular glaucoma [11]. Approximately 50 percent of patients with severe NPDR progress to proliferative retinopathy within one year [12].

2.2. Diabetic retinopathy is a vascular-neuroinflammatory disease

The early signs of DR include vascular inflammatory reactions as indicated by increases in release of cytokines, increased expression of the leukocyte adhesion molecules CD18 and intercellular adhesion molecule 1 (ICAM-1), breakdown of the blood-retinal barrier (BRB) function and loss of retinal neurons [13–19]. The retina may be damaged by hyperglycemia-induced formation of reactive oxygen species (ROS), including superoxide and peroxynitrite [20,21]. While it is not yet clear whether the excessive ROS either first damages vascular or neuronal tissues of the retina, both microglial and macroglial cells are activated [22]. The function of activated macroglia in transporting [23] and metabolizing glutamate [24] may be impaired. This may lead to glutamate accumulation [25–27], activation of N-methyl-D-aspartic acid (NMDA) receptors, calcium influx, formation of superoxide and peroxynitrite, and neuronal death [28]. Activated microglia release cytotoxic molecules in response to cytokines to further exacerbate the damage [29]. Thus, whereas the retina has traditionally been viewed as an immune privileged tissue, evidence is accumulating to support a role for local inflammation in the pathogenesis of DR [15,18,30,31]. These molecular and clinical observations collectively suggest that novel pharmacological interventions that reduce oxidative stress and inflammation may be effective neuroprotectants for DR [27,32].

3. Cannabidiol as a potential therapy for diabetic retinopathy

The marijuana-derived cannabinoids (−)-Δ9–tetrahydrocannabinol (THC) and (−)-cannabidiol (CBD) both have anti-oxidative [33,34] and immunosuppressive effects [35–37]. The CB1 and CB2 cannabinoid receptors mediate the psychotropic and anti-inflammatory effects of THC, respectively [36,38]. CBD does not, however, show strong affinity to these receptors [39]. This low affinity results in the inability of CBD to produce the subjective "high" and cognitive effects that are characteristic of marijuana and THC [40,41]. On the other hand, CBD is very effective as a scavenger of ROS. The antioxidative effect of CBD is superior to *α*-tocopherol and ascorbate *in vitro* and *in vivo* [33], due to its ability to scavenge ROS and block NADPH oxidase [42]. In experimental cellular or animal models, CBD has potent anti-inflammatory actions and have been shown to decrease inflammatory cytokines in arthritis [43] and in diabetes [17], prevent cerebral damage during ischemia [44], and to prevent cerebral infarction [45]. CBD attenuates high glucose-induced endothelial cell inflammatory response and barrier disruption in human coronary endothelial cells [46]. It also decreases the incidence of diabetes in non-obese diabetic mice [47], and is neuroprotective and BRB-preserving in streptozotocin-induced diabetes [17]. Most recently, CBD has been shown to decrease retinal inflammation by blocking ROS and TNF-α formation, p38 MAP kinase activation, and microglial activation [42]. Currently, our preclinical studies have provided evidence for the anti-inflammatory effects of intraocular CBD in the rat model of DR. In humans, CBD is well tolerated when chronically administered [48], and has been approved for the treatment of inflammation, pain, and spasticity associated with multiple sclerosis since 2005 [49]. However, CBD's potential clinical efficacy and limitations in patients with diabetes have not been tested thus far.

3.1. CBD enhances the anti-inflammatory effects of adenosine in the retina

Adenosine has been proposed to modulate a variety of physiological responses by stimulating specific extracellular receptors [50–52]. Adenosine receptors (ARs) have been classified as A_1, A_{2A}, A_{2B}, and A_3 receptors [53]. Under stress and ischemia conditions, the local tissue concentrations of the extracellular adenosine are increased due to the release of adenosine itself, or of AMP, which is metabolized extracellularly to adenosine. Adenosine released at inflamed sites exhibits anti-inflammatory effects through the A_{2A}AR [54]. Interestingly, sub-threshold doses of an inflammatory stimulus that caused minimal tissue damage in wild-type mice were sufficient to induce extensive tissue damage and more prolonged and higher levels of pro-inflammatory cytokines in knock-out mice that lacked the A_{2A}AR (A_{2A}AR −/− mice) [55]. A_{2A}AR agonist treatment blocked the inflammation, functional and histological changes associated with diabetic nephropathy in wild-type diabetic mice, whereas it had no effect on the A_{2A}AR −/− diabetic mice [56]. A_{2A}AR, a Gs-protein-coupled receptor, can increase levels of immunosuppressive cAMP in microglia or other immune cells [57]. Stimulation of the A_{2A}AR decreases leukocyte adhesion and blocks the associated release of oxygen free radicals [58]. Adenosine released can activate endothelial ARs, leading to increases in intracellular cAMP and resealing of the endothelial junctions, thereby promoting vascular barrier function [59]. Moreover, A_{2A}AR activation induces both synthesis and release of nerve growth factor thereby is neuroprotective [60].

Although adenosine and its agonists are protective in animal models of inflammation, their therapeutic application has been limited by systemic side effects, such as hypotension, bradycardia, and sedation [61]. Adenosine usually disappears very rapidly in physiological or inflammatory conditions due to rapid reuptake via nucleoside transporters (NTs) and subsequent intracellular metabolism [62]. Because adenosine levels at inflamed sites increases, prevention of adenosine reuptake and metabolism can selectively enhance extracellular concentrations of adenosine at inflamed sites, resulting in a site-specific anti-inflammatory effect [63]. Protective or ameliorating effects of adenosine uptake inhibitors in ischemic cardiac

and cerebral injury, organ transplantation, seizures, thrombosis, insomnia, pain, and inflammatory diseases have been reported [64]. Hence, preclinical and clinical results indicate the promise of therapeutic application of adenosine uptake inhibitors [64,65]. To this end, there are two subtypes of NTs:

- concentrative NTs, which are dependent on the presence of extracellular sodium and,
- equilibrative NT (ENTs).

In the microglial cells, the majority of adenosine transport is not affected by sodium removal, suggesting ENTs are the primary transporters functioning in these cells [66]. ENTs are classified into two subtypes on the basis of their sensitivities to inhibition by the drug nitrobenzylmercaptopurine riboside (NBMPR). NBMPR-sensitive ENTs bind NBMPR with high affinity and have the functional designation equilibrative sensitive (ENT1). NBMPR-insensitive transporters are designated ENT2. Dipyridamole, an inhibitor for both ENT1 and ENT2 [67,68], is used clinically as a coronary vasodilator and a platelet aggregation inhibitor [69,70]. Dipyridamole plus aspirin improves retinal vasculature patterns in experimental diabetes [71]. ENT1 plays an integral role in adenosine function in diabetes by regulating adenosine levels in the vicinity of adenosine receptors [72]. In the latter study, adenosine uptake by ENT1 in human aortic smooth muscle cells (HASMCs) was increased by hyperglycemia [72]. To provide insight into mechanisms by which ENT1 was modulated by hyperglycemia, kinetic studies of adenosine transport and [^{3}H]NBMPR binding were performed [72]. The results show that *V*max (representing the number of ENT1) of adenosine transport in high glucose (HG)-treated HASMCs was increased without affecting *K*m (representing the affinity of ENT1). Similarly, *B*max (representing the number of ENT1) of the high-affinity [^{3}H] NBMPR binding was increased without affecting *K*d (representing the affinity of ENT1). Consistent with these observations, HG increased mRNA and protein expression of ENT1 [72]. From a pathophysiology standpoint, the increase in ENT1 activity in diabetes may affect the availability of adenosine in the vicinity of adenosine receptors and, thus, alter vascular functions in diabetes.

It has recently been shown that the nanomolar concentrations of CBD or THC could inhibit uptake of adenosine by ENT1 in murine microglia, RAW264.7 macrophages [66], and in rat retinal microglia [35]. CBD synergistically enhances adenosine's TNF-α suppression upon LPS treatment [35]. Moreover, *in vivo* treatment with a low dose of CBD decreases TNF-α production in serum in the LPS-treated mice; this effect is reversed by treatment with an A_{2A}AR antagonist and abolished in A_{2A}AR −/− mice [66]. Similar results are observed in the rat retina [35]. These studies collectively demonstrate that CBD has the ability to enhance adenosine signaling through inhibition of uptake via ENT1 and provide a non-cannabinoid receptor mechanism by which CBD can decrease endotoxin-induced inflammation. Current results suggest that CBD inhibits diabetes-induced retinal inflammation by the same mechanism (Unpublished observations).

4. Biomarker development strategies for targeted clinical development of CBD in patients with diabetic retinopathy

The lessons learned from past drug development efforts in DR tell us that a more rational strategy is required to discover and test novel drug candidates as well as the need for better transition from preclinical to clinical first-in-human studies. To these ends, we present a hypothetical pathway (summarizing the discussions above) illustrating how CBD exerts its pharmacodynamic action to reduce retinal inflammation in diabetes (Figure 1). The elements in this figure can serve as a useful foundation to map the future biomarker development strategies in clinical proof of concept studies of CBD treatment of DR.

Insofar as the putative genetic markers are concerned, a hereditary component has been reported both for the prevalence and severity of DR [73–75]. A number of candidate genes encoding proteins involved in aldose reductase pathway, major histocompatibility complex and immunity, glucose transporters, cell communication and the extracellular matrix, endothelial function and nitric oxide synthases have been studied [76,77] (Table 1). Thus far, these findings do not always corroborate each other but this is also a commonly observed scenario in candidate gene studies that will still demand future replication efforts, to account for variations in characteristics of study populations, substructures created by molecularly heterogenous phenotypic representations and classification of DR severity, among other confounding factors. Moreover, candidate drug targeted pathway-related genetic variants add up to this challenge considering the multi-factorial nature of the studied phenotype (i.e., phenotypes on drug efficacy and toxicity). Genome-wide association studies in well characterized patient populations evaluated with standardized DR severity grading system will be essential to evaluate the findings from candidate gene studies published to date. These broader genome wide inquiries can also inform to establish the link between postulated molecular mechanisms and clinical pharmacodynamics of CBD after administration in patients with DR.

In regards to drug metabolism and distribution, it is also important to bear in mind the pertinent biomarker pathways that can potentially impact the CBD concentrations in the systemic circulation or locally in the retina [78,79]. Importantly, the phase I metabolism of the classical cannabinoids (including THC, cannabinol and CBD) has been shown to depend primarily on the cytochrome P450 mixed-function oxidases, CYP2C9 and CYP3A4 in human hepatic microsomes [79]. The impact of the *CYP2C9* polymorphisms on the clinical pharmacokinetics of orally administered THC was studied in 43 healthy volunteers [80]. The results revealed that subjects carrying a coding variant (Ile359Leu) (*CYP2C9*3*) had decreased total clearance for THC and may thereby express enhanced therapeutic and adverse effects of orally administered THC. *CYP2C9*3* encodes an enzyme with 3–30 fold lower activity in comparison to the wildtype (*CYP2C9*1*) depending on the specific substrate [81]. Although the quantitative contributions of CYP2C9 and CYP3A4 enzymes to CBD metabolism have not been elucidated, based on the structural similarity of THC, cannabinol and CBD, the impact of the *CYP2C9* polymorphism on the potential pharmacokinetics of orally administered CBD can potentially be anticipated.

In contrast to the phase I metabolism, very little is known about the phase II metabolism of classical cannabinoids [82]. Phase II metabolites appear to be mainly conjugates of the phase I metabolites with glucuronic acid, catalyzed by the activity of glucuronosyltransferases. The conjugates are the main metabolites of THC or CBD found in urine [83].

In addition to the potential impact of genetic variation of phase I and phase II enzymes on CBD metabolism, the inhibitory/inductive effect of CBD on phase I and phase II drug metabolism activity and the role of genetic variation in baseline (constitutive) drug metabolizing enzyme activity should be considered with respect to the (prediction) of the risk for drug-drug interactions with CBD during its future development. To this end, CBD has been shown to inhibit CYP2C and CYP3A enzyme activities in rat and mouse hepatic microsomes [84].

Ocular pharmacokinetics of a drug is another potential challenge to consider in personalized/targeted drug development as both systemic and local administration routes might involve a genetically determined interindividual variability. Corneal epithelium and blood-retina barrier constitutes the primary regulating sites of xenobiotic access into ocular tissues. Moreover, the presence of both phase I and phase II drug metabolizing enzymes and transporter proteins, including organic anion and cation transporters as well as efflux proteins, have been shown in several ocular tissues in different ranges, retinal pigment epithelium and ciliary body being the

primary detoxification cites for xenobiotics [85,86]. The impact of genetic variation of these components on ocular drug pharmacokinetics has not been studied yet and this aspect certainly requires substantial thoughtful attention in future ocular drug development in relation to efficacy, dose finding and safety.

Insofar as the primary molecular targets for CBD (for applications in DR) and biomarker development are concerned, inhibition of adenosine reuptake via ENT1 in rat microglia is postulated as the mechanism of action for the anti-inflammatory effects of CBD [35] (Figure 1). The gene encoding the human ENT1, *SLC29A1*, is mapped to chromosome 6p21.1-21.2 and several polymorphisms have been identified in the promoter and coding regions of the gene [87–89]. Notably, a 1.37-fold increased *in vivo* (white blood cells) expression of the transporter has been shown for individuals heterozygous for the variant -1345C/ -1050G/ -706C haplotype compared to individuals homozygous for the wild-type -1345C/ -1050G/ -706G haplotype [88]. However, these results obtained from white blood cells may not reflect the activity of the ubiquitously expressed *ENT1*. A potential interindividual difference in ubiquitous or ocular *ETN1* expression in relation to these haplotypes deserves further investigation.

5. Conclusions and Future Outlook

Despite past disappointments on lack of effective or clinically acceptable treatments for DR, a disease with global public health significance, recent mechanistic evidence suggests new insights and that local inflammation plays a major role in its pathogenesis [15,18,30,31]. The function of CBD as an antioxidant to block oxidative stress and as an inhibitor of adenosine reuptake to enhance a self-defense mechanism against retinal inflammation, as suggested by our preclinical observations, theoretically represents a novel therapeutic approach for the diabetic complications of the eye. However, the therapeutic effectiveness of this agent is not presently confirmed by clinical trials, and it is unknown whether CBD can be developed with personalized medicine strategies to offer significant advantages over traditional clinical drug development. Nevertheless, the analysis presented herein emphasizes the need for rational *a priori* preclinical conceptual considerations on the mode of action of CBD in DR treatment which, by extension, can help future biomarker discovery and development efforts in the clinic.

So far, decision-making for the treatment choice and dosage has been applied in clinical practice mostly in a trial-and-error approach using available patient information, including age, gender, organ dysfunctions, clinical disease subtype, medical history, and familial history of hereditary diseases. The developments in pharmacogenetics and pharmacogenomics provide new avenues for biomarker guided drug prescription and better utilization of preclinical data to inform targeted clinical drug development. The pathways presented in Table 1 and Figure 1 can help focus efforts to identify biomarkers of CBD effects in clinical studies. Additionally, the pharmacokinetic pathways that might contribute to CBD metabolism and genetic variation in *SLC29A1* represent some of the actionable candidate biomarker pathways for CBD. As a complement to genomics, an integrative approach using other biomarker technology platforms based on metabonomics and proteomics might be essential in order to validate biomarkers for DR susceptibility and therapeutic response to CBD in DR.

Using information obtained from pharmacodynamic and pharmacokinetic biomarkers in early clinical proof of concept studies may allow stratifying patients according to their potential responsiveness and dose requirement and prevent a failure at this stage due to lack of efficacy or adverse events. Moreover, such an approach in further clinical development studies would potentially decrease the number of test subjects needed and reduce the development costs. Safety of an ophthalmologic drug candidate for use in patients with DR might require particular attention due to limitations in drug elimination from ocular compartment.

Personalized medicine promotes the use of biomarkers to predict patients who are more likely to benefit from a certain therapy and/or identification of patients at higher risk for adverse effects during each treatment. On the other hand, limiting the size of potential target population before launching a novel drug is often met with ambivalence by commercial stakeholders, and therefore the application of personalized medicine is still a challenging task for drug developers, the insurers or the health systems that will in the future rely on personalized medicines. Initiating and planning biomarker development efforts early on in drug development and integrating preclinical observations will remain as essential ingredients of a targeted/personalized medicine research and clinical evaluation strategy. Moreover, we suggest that demands for comparative effectiveness of new drugs may further catalyze the need for and interest in personalized medicines in ophthalmology and other medical specialties [90]. Understanding the biological action of cannabidiol in preclinical studies as an integral part of clinical biomarker development also contributes towards efforts to allow advances at the hitherto neglected intersection of personalized medicine and ophthalmology.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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List of abbreviations

AR	adenosine receptor
BRB	blood-retinal barrier
CBD	cannabidiol
DR	diabetic retinopathy
ENT	equilibrative nucleoside transporter
LPS	lipopolysaccharide
PDR	proliferative diabetic retinopathy
ROS	reactive oxygen species
SNP	single nucleotide polymorphism

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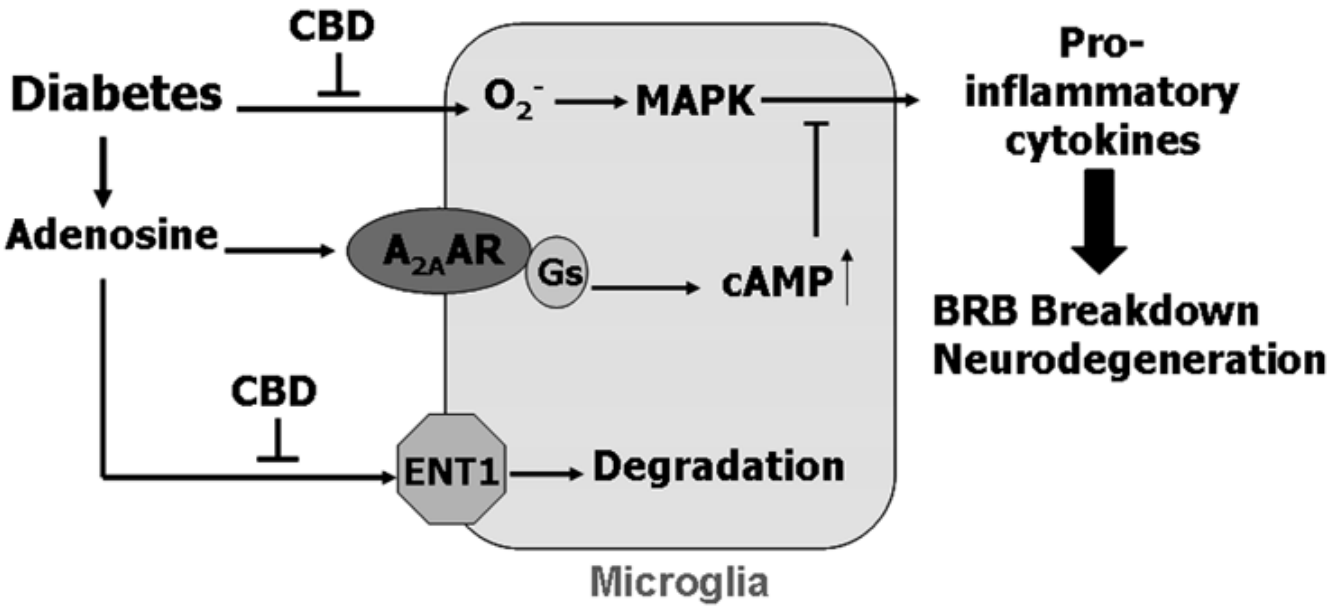

Figure 1. The postulated anti-inflammation mechanism of action by CBD in treatment of DR

Diabetes causes release of adenosine and pro-inflammatory cytokines via superoxide formation and MAPK activation, leading to DR. Adenosine-initiated anti-inflammation via A_{2A}AR-Gs-cAMP signaling is terminated rapidly due to adenosine reuptake by equilibrative nucleoside transporter (ENT) and subsequent metabolism and degradation. CBD blocks superoxide formation. CBD also inhibits adenosine reuptake via inhibiting ENT1, thereby activating A_{2A}AR-Gs-cAMP signaling.

Table 1

Candidate genes studied in relation to susceptibility for diabetic retinopathy.

Pathway	Gene	Mechanism
Aldose reductase	*AR2 Aldose reductase*	Altered microvascular stability
Major Histocompatibility Complex and immunity markers	*HLA, Human leukocyte antigen* *IgG, Immunoglobulin G* *IgM, Immunoglobulin M* *IL6, Interleukin 6* *TLR4, Toll-like receptor 4*	Altered innate immunity
Glucose transporters	*GLUT1, Glucose transporter 1*	Altered glucose transport and regulation
Cell communication and the extracellular matrix	*PAI-1, Plasminogen activator inhibitor 1* *APOE, Apolipoprotein E* *TNF-α, Tumor necrosis factor alpha* *β-3AR, Beta 3 adrenoreceptor* *PON1, Paraoxonase 1* *α2β1I, Alpha 2 beta 1 integrin collagen IV α1* *Gβ3, G-protein beta 3 subunit* *NPY, Neuropeptide Y* *ICAM1, Intercellular adhesion molecule 1* *VEGF, Vascular endothelial growth factor*	Altered angiogenesis
Endothelial function and nitric oxide synthases	*ACE, Angiotensin-converting enzyme* *AGTII-IR, Angiotensin type 1 receptor* *AGT, Angiotensinogen* *NOS2A, Inducible nitric ixide synthase* *NOS3, Constitutive nitric oxide synthase* *EDN1, Endothelin 1* *MTHFR, Methylenetetrahydrofolate reductase* *MTRR, 5-methyltetrahydrofolate-homocysteine methyltransferase reductase*	Altered regulation of endothelium-mediated vascular flow