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Revisión

Cannabinoid applications in glaucoma

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A B S T R A C T

Introduction: Glaucoma is a slowly progressive optic neuropathy that is one of the leading causes of legal blindness throughout the world. Currently there is a limited group of topical drugs for the medical treatment of glaucoma is currently limited, and research needs to be focused on new therapeutic horizons, such as the potential usefulness of the cannabinoid agonists for the treatment of glaucoma.

Aim: To review the current scientific literature related to the beneficial effects derived from the different ways of administration of cannabinoids indicated for the glaucomatous optic neuropathy.

Development: Cannabinoid receptors have shown an intense expression in ocular tissues implicated in the regulation of the intraocular pressure, as well as inner layers of the retina. Through activation of CB1 and CB1 specific receptors and through other still unknown pathways, the cannabinoid agonists have shown both a clear hypotensive, as well as an experimentally proved neuroprotective effect on retinal ganglion cells.

Conclusions: Some cannabinoid agonists (WIN 55212-2, anandamide) have demonstrated, in experimental studies, to act as «ideal drugs» in the management of glaucoma, as they have been shown to have good tolerability after topical application, efficiently reduce intraocular pressure, and behave as neuroprotectors on retinal ganglion cells.

Further studies as regards the safety and clinical assays must be carried out in order to examine the effectiveness of these drugs for the treatment of glaucoma in our daily clinical practice.

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Aplicaciones de los cannabinoides en glaucoma

Palabras clave:
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RESUMEN

Introducción: El glaucoma es una neuropatía óptica lentamente progresiva que constituye una de las principales causas de ceguera legal en el mundo. Actualmente existe un limitado grupo de fármacos tópicos para su manejo médico, siendo necesario enfocar la investigación hacia nuevos horizontes terapéuticos como el potencialmente útil grupo de los agonistas de cannabinoides.

Objetivo: Revisar a través de la literatura científica actual, los efectos beneficiosos a través de distintas vías de administración de los cannabinoides para la neuropatía óptica glaucomatosa.

Desarrollo: Los receptores de cannabinoides han demostrado una amplia expresión en los tejidos oculares implicados en la regulación de la tensión ocular, así como en las capas internas de la retina. Mediante la activación de receptores específicos CB1, CB2 y vías aún no bien conocidas, los agonistas de cannabinoides han demostrado un claro efecto hipotensor ocular, así como un probado efecto neuroprotector sobre las células ganglionares de la retina en estudios experimentales.

Conclusiones: Algunos cannabinoides (WIN 55212-2, anandamida) han demostrado a nivel experimental actuar como «fármacos ideales» en el manejo del glaucoma, al presentar buena tolerancia tras su aplicación tópica, reducir de forma eficaz la presión intraocular, y presentar un probado carácter neuroprotector sobre las células ganglionares de la retina.

Se deben realizar más estudios sobre su seguridad y ensayos clínicos para poder examinar la utilidad de estos fármacos en el tratamiento del glaucoma en nuestra clínica diaria.

Introduction

In numerous studies, cannabinoids have demonstrated beneficial effects, increasing neuron survival in neurodegenerative diseases, evidencing various ways through which cannabinoids express their neuroprotective effect.[1-6]

The applicability of cannabinoids in ophthalmology has the main objective of treating various retinal neurodegenerative diseases (Leber optic neuropathy, dominant optic atrophy and glaucoma, among others). Even though the origin and evolution of these diseases is different, we find common pathways through which the retinal ganglionary cells are damaged, and precisely through these mechanisms and by means of controlling the various risk factors we can act to slow down their progression.

Glaucoma is one of the main causes of legal blindness in the world and the most prevalent retinal neurodegenerative disease. A large number of studies and research has been made in the field of cannabinoids as neuroprotective agents.

Said neuroprotective effects, associated to the research which was started in the 70s by Hepler et al,[7] demonstrated the reduction of the intraocular pressure after inhaling marihuana and gave rise to an increasing amount of studies to verify the usefulness of various cannabinoid compounds for treating glaucoma.

Objective

To describe the involvement of the endogenous endocannabinoid system in the physiopathology of glaucoma.

In addition, this review aims at assessing the main evidence described in scientific literature concerning the beneficial role of cannabinoids in glaucomatous optic neuropathy due to its influence in controlling intraocular pressure as well as its neuroprotective role in secondary degeneration that begins with glaucoma.

Development

Glaucoma

Chronic primary open angle glaucoma (CPOAG) is a slowly progressive optic neuropathy which induces structural changes in the optic nerve, clinically related to a visual fields loss. The death of retinal ganglion cells, the most representative characteristic of glaucoma, occurs in two stages. In the first, influenced by the main risk factor (intraocular hypertension), it induces an alteration of the correct trophism of the retina ganglionary cells. According to the mechanical and vascular theories, the increased intraocular pressure stimulates a chain of events which induces apoptosis of the

Table 1 – Immunohistochemical marking of CB1 receptors at the ocular level

Structure	Marking
Anterior segment	
Corneal epithelium	+++++
Corneal stroma	–
Corneal endothelium	+++++
Trabecular mesh	+++++
Trabecular epithelium	+++++
Schlemm's canal	+++
Uveal tract	
Iris anterior edge	–
Stroma	–
Pigmentary epithelium	–
Iris base	–
Ciliary muscle	+++++
Nonpigmented ciliary epithelium	+++++
Pigmented ciliary epithelium	–
Blood vessels in ciliary body	+++
Retina	
Choroids	–
Retina pigmented epithelium	–
Photoreceptor external segments	+++++
Photoreceptor internal segments	++++
External plexiform layer	+++
External nuclear layer	++
Internal plexiform layer	+++
Internal nuclear layer	+++
Retina ganglion cell layer	+++
Nervous fiber layer	+++

Immunohistochemical marking of CB1 receptors at the ocular level.
–: Absence of marking; +: slight marking; ++: slight – moderate; +++: moderate; ++++: moderate-intense; +++++: intense.
Table based on the data of the article by Straiker et al, 1999.

retina ganglionary cells.[8] The production of free radicals as well as the neurotoxicity of nitric oxide and the excitotoxicity regulated by glutamate enhance the initial effects of the lesion and facilitate the development and progression of glaucoma.[9] It has been postulated that this secondary environment increases the progression of glaucomatous damage. This secondary neurodegeneration must be acted upon when developing a neuroprotective strategy against glaucomatous optic neuropathy.

Accordingly, the best drugs to utilize in treating glaucoma are those which, applied topically in the absence of systemic side effects, have the ability of penetrating the target ocular tissue and controlling the main risk factor for the development of glaucomatous damage (ocular hypertension) and which in addition develop a neuroprotective effect on the retina ganglionary cells.

Cannabinoids

The cannabis plant has over 400 chemical components and 60 cannabinoids.[10] Cannabinoids are substances which generally have a 21-carbon carbocyclic structure generally made up by three rings of cyclohexane, tetrahydropirane and benzene.

The main psychoactive element of cannabis is Δ9-tetrahydrocannabinol (Δ9-THC), the structure of which was defined in the 60s.[11]

Other relevant cannabinoids are Δ8-tetrahydrocannabinol (Δ8-THC), cannabidiol (CBD), cannabinol (CBN), cannabichromene (CBC), cannabicyclol (CBL), cannabigerol (CBG), cannabigerol monomethylether (CBGN), cannabielsoine (CBE), cannabinodiole (CBND), cannabitriol (CBT), dehydrocannabifurane and cannabicytrane.[12]

Endogenous ocular cannabinoid system

Definition and main endocannabinoids

Endocannabinoids are long chain fatty acid amides and esters. Anandamide (AEA) and 2-acyl-glycerole (2-AG) are the most widely studied endocannabinoids. The group of endocannabinoids, the receptors to which they join and the proteins they synthesize, transport and hydrolyze is known as the "endogenous endocannabinoid system".[13]

Numerous studies have been made on the endocannabinoids system in the eye. The presence, synthesis and degradation of AEA has been evidenced in various ocular structures of different mammals in porcine,[14] bovine[15] and murine[16] models and in humans.[17] On the other hand, the presence of the main receptor subtypes CB1 and CB2 has been demonstrated in rat retinas, as well as vanilloid receptors with which certain cannabinoid compounds exhibit affinity.[18]

In addition, an increasing number of scientific observations indicate that endocannabinoids are relevant to ocular physiology and intervene in maintaining intraocular pressure[19,] in the physiology of photo reception and neurotransmission in the retina[20,] as well as in neuroprotection.[21]

Cannabinoid receptors

Two cannabinoid receptors have been pharmacologically cloned and characterized (CB1 and CB2)[22,23] although, as mentioned above, some cannabinoids exhibit affinity with vanilloid receptors, and mounting evidence proves the existence of non- CB1/CB2 cannabinoid receptors.[24]

Localization of cannabinoid receptors at the ocular level

Although the expression of CB1 and CB2 has been described in ocular tissues, the main cannabinoid receptors at the ocular level are CB1. A study on rats eyes *in toto*[25] demonstrated the presence of CB1 receptor messenger RNA in ocular tissues. Subsequently, the distribution of CB1 receptors was described in human eyes which had been preserved *postmortem* in paraffin (table 1).[26] Intense markings were detected in the corneal epithelium, endothelium, the ciliar epithelium and photoreceptor external segments. This marking was moderate-intense in the trabecular mesh and the Schlemm canal, moderate at the level of the ciliary body blood vessels, ciliary muscle and internal and external plexiform layers, as well as on the internal nuclear layer and the retinal ganglion cells layer, demonstrating specific markings in the bipolar, amacrine and horizontal cells.[27] The marking was moderate to slight in the pupil sphincter. It is also interesting to note the detection of functional cannabinoid receptors at the level of bovine ophthalmic arteries.[28]

Table 2 – The Main studies on the topical application of cannabinoids

Autor	n	Molec.	Adm.	[]	Effect	Side. effect
Oltmanns et al, 2008 [38]	Rats Sprague-Dawley HTO x CVE	WIN 55212-2	Topical (Tocrisolve™)	1%, 0.25%, 0.06%, 0.0015%	↓ IOP >120ï at 1%: 30ï: ↓32% 120ï: ↓52%.	No
Tomida et al, 2006 [32]	6 humans OACG/HTO	Δ-9-THC CBD	Subling.	Δ-9-THC (5 mg) CBD (20/40 mg)	Δ-9-THC: Temporary reduction of IOP CBD: 20 mg: No effect on IOP 40 mg: Temp. increase of IOP	Moderate transient anxieyt crisis (n=1)
Hosseini et al, 2006 [71]	Rats Sprague-Dawley normotensive (14.1±0.7)	WIN 55212-2	Topical (Tocrisolve™)	20µl 0.5%	↓ 47% IOP baseline (6.6±0.2mm Hg)	No
Porcella et al, 2001 [34]	Humans OACG (IOP > 22mm Hg)	WIN 55212-2	Topical (45% 2-h-β-CDO)	25-50µl	Maximum effect at 60ï 25µl: ↓IOP 20±0.7% 50µl: ↓IOP 31±0.6%	No
Pate et al, 1998 [72]	NZW and Dutch Belted pigmented rabbits	CP-55.940 (1mg/ml) AEA (2.5 mg/ml)	Topical (20%-2-h-β-CDO+3% polyvinyl alcohol)	25µl AEA: 62.5µg 25µl CP-55940: 25µg	↓ IOP	No
Pate et al, 1996 [73]	NZW and Dutch Belted pigmented rabbits	AEA	Topical (5-30% 2-h-β-CDO)	25µl a: 1.25 mg/ml 2.5 mg/ml	↑ initial and posterior IOP ↓ (slight contralateral effect): 60':↓ 3.5±0.5mm Hg 120': ↓5.2±1.3mm Hg	No
Green et al, 1982 [74]	Humanos V.S.	Δ-9-THC	Tópico	1 gota	No effect on IOP	Midriasis, pruritus, tearing

[]: concentration; 2-h-β-CDO: 2-hydroxypropyl-β-cyclodextrine; AEA: anandamide; Side eff.: Side effects; Δ9-THC: Δ9-tetrahydrocannabinol; OACG: open angle chronic glaucoma; Molec.: Utilized compound; n: sample number and/or type; IOP: intraocular pressure;wk.: week; subling.: sublingual.

Ophthalmological effects of cannabinoids

Cannabinoids and intraocular pressure

Different cannabinoid compounds have demonstrated the reduction of intraocular pressure through different administration pathways, as described for inhaled Δ9-THC[7,29,] oral[30,] intravenous[31,] sublingual[32] and after topical administration at the ocular level[33,34] (table 2).

Although the exact mechanism by which cannabinoids are able to regulate ocular pressure is not known, an intense marking of CB1-type cannabinoid receptors has been identified in locations involved in the production and excretion of the aqueous humor, including the ciliary body, its blood vessels, the ciliary muscle and the trabecular mesh.[26]

The presence of intense markings for CB1-type receptors at the level of the non-pigmented epithelium of the ciliary body and in the choroidal vessels defines one of the main mechanisms through which cannabinoid agonists could lower intraocular pressure by diminishing the production of aqueous humor.

Said marking for CB1 receptors is also intense at the level of the ciliary muscle, which has a basic defect for increasing the filtration of aqueous humor through the uveoscleral pathway,[35] while being able to modify at the same time the arrangement of the trabecular mesh. On the other hand, a sustained contraction thereof could diminish the range of accommodation, a phenomenon observed in subjects under the effects of inhaled marihuana.

Some studies consider that the main hypotensive mechanism is the increased ease of excretion of the aqueous humor after verifying that it doubled or even tripled without registering a reduction in the production thereof after the topical and systemic application of Δ9-THC and CBG[36] by producing an increase in the dimensions of Schlemm's canal.[34] Treatment with noladin ether (endocannabinoid agonist) induces an activation of kinase metaloproteinkinase P42/44, giving rise to a remodeling of the trabecular mesh cells with increased sphericity and diminishing the production of actin stress fibers and focal adhesions. These effects are blocked by the antagonist of CB1 receptors SR141716A or rimonabant.[37]

It appears that the cannabinoids induce said ocular hypotension mainly through the CB1 receptors. Oltmanns et al described a clear ocular hypotensive effect of the WIN 55212-2 agonist at 0.5% and 1% after dissolving in Tocrisolve™.

The duration of this effect was significantly diminished after previously applying the antagonist of CB1 receptors SR 141716A.[38]

However, the effect of CB2 receptors in diminishing ocular pressure is not yet very well determined because the topical application of CB2 receptors (SR 144528) has not demonstrated its ability to inhibit the topical hypotensor effect of WIN 55212-2[38] to the same extent as CB1 antagonists such as rimonabant.

However, the topical indication of dexanabinol (HU-211) at 0.12%, one of the most powerful non-psychoactive synthetic cannabinoids described to date,[39] produces a significant reduction of intraocular pressure in normotensive rabbits[33] with a duration of 6 hours and an ocular hypotensor effect in the contralateral eye for hours after administering the drop. The intravenous administration of HU-211 induces a dosage dependent reduction of intraocular pressure greater than Δ^9-THC and el Δ^8-THC.[40] Its effect is inhibited by yohimbine (α-2-adrenergic antagonist) and propanolole (β-adrenergic antagonist). In addition, it lacks affinity with CB1 or CB2 receptors, which suggests that its ocular hypotensive effect could occur through other pathways than those dependent of the main cannabinoid receptors[41].

Cannabinoids and neuroprotection

Numerous studies have demonstrated the neuroprotective effect of cannabinoids in central nervous system neurodegenerative diseases such as Parkinson's.[1] Alzheimer,[2] multiple sclerosis[5] and Huntington's disease.[6]

The neuroprotective effect of cannabinoids occurs through different action mechanisms.[42] Accordingly, the activation of presynaptic CB receptors inhibits in retrograde manner the release of glutamate, improving the control of neuronal excitability and regulating synaptic plasticity.[42,43] Its activation also induces an increased expression of the brain-derived neurotrophic factor (BDNF), also increasing neuronal survival through neuromodulation mechanisms in the oligodendroglyal cells.[44,45] In turn, the activation of CB2 receptors performs its neuroprotective effects modulating neuronal inflammation through the microglya, macrophages and dendritic cells, also increasing the autocrine production of endocannabinoids (AEA, 2-AG), as demonstrated in multiple sclerosis patients.[46] Similarly, there is evidence of the neuroprotective effect of endocannabinoids (AEA) both *in vivo* as in *in vitro* through non-CB1/CB2 receptors.[21,47,48] On the other hand, other cannabinoids (HU-211) have demonstrated neuroprotective effects by directly blocking the excitotoxicity of glutamate – induced toxic pathway through the NMDA receptors.[49]

Glutamate – induced excitotoxicity inhibition. In glaucoma, the intravitreal levels of glutamate are increased.[50,51] Glutamate, an excitatory neurotransmitter either through the activation of NMDA or non-NMDA receptors, increases the intracellular calcium levels, inducing lipidic peroxidation and increased oxidative stress through nitric oxide and nitrogenated free radicals.[52] Said excitotoxicity has demonstrated toxic effects on the retina, particularly on the larger size retinal ganglion cells[53-55] which are affected in the early stages of glaucoma.

Some cannabinoids have demonstrated a new neuroprotective effect on the retina ganglion cells submitted to oxidative stress[56,57] or in glutamate-mediated excitotoxicity models by inhibiting the formation of nitric oxide after an intravitreal injection of NMDA, as is the case of Δ9-THC at a dose of 0.4 and 2mg/kg, of which the dosage-dependent effect was partially blocked by the rimonabant antagonist, which places this neuroprotective mechanism at the level of the CB1- type receptors.[52] These CB1 receptors could play a neuroprotective role by inhibiting the voltage-dependent calcium channels[57]. However, it is not clear that the new neuroprotective effects supplied by cannabinoids remain exclusively at the level of the CB1 receptors, as the use of CBD, a non-psychotropic cannabinoid which does not activate the CB1 receptors, also demonstrated *in vivo* neuroprotective effects by preventing the formation of nitrotirosine.[52] In addition, CBD does not only have neuroprotective effects *per se*; it also inhibits the degradation of the AEA endogenous cannabinoid.[58]

Beneficial vascular effects on the optic nerve. As early as 1998, CB1 receptors were demonstrated in smooth muscular fibres and aortic endothelials[59] and subsequently at the level of the ophthalmic bovine arteries.[28]

There is an increasing number of clinical studies on the vascular flow at the level of the optic papilla which consider the reduction of vascular flow as one of the fundamental mechanisms regulating the physiopathology of glaucoma. The density of the capillaries that irrigate the optic disc in glaucomatous eyes are similar to controls.[60] However, CPOAG patients exhibit a lower flow at the level of the optic nerve head without statistically significant differences being found between patients with isolated ocular hypertension and controls.[61]

Cannabinoid agonists produce vascular relaxation through the activation of K+ channels through the GMP-c and nitric oxide pathways.[28] AEA and WIN 55212-2 produce a dosage dependent vasodilator effect through endothelium-derived relaxing factors such as nitric oxide,[62] by the stimulation of CB1 receptors and vanilloids.[28]

The deleterious effects described on retinal and optic nerve circulation derived form the data obtained after inhaling Δ9-THC when smoking cigarettes (reduction in systolic and diastolic arterial pressure and tachycardia)[63] were subsequently refuted because of the oral ingestion of dronabinol demonstrated through fluorescein angiography an increase of retinal perfusion in healthy individuals without demonstrating adverse effects at the cardiovascular or respiratory level.[64] This effect on retinal circulation differs from a prior experimental study on rabbits which did not demonstrate an increase in retinal vascular flow although an increase in the circulation in the iris, the ciliary body and the choroids was determined.[65]

Topical application of cannabinoids

Topical application, which is further away from possible systemic side effects associated to other administration pathways, is the pathway to be taken into account in future applications for treating glaucomatous optic

neuropathy.[66] Due to its high liposollubility and the need of utilizing lipophyllic products for adequate dissolution, numerous vehicles such as ethanol, dimethyl sulfoxide, polivynilpyrrolidone, Tween 80. cremofor, emulfor, bovine serum albumina (BSA), 2-hydroxypropyl-β-cyclodextrine, and recently the utilization of Tocrisolve™[67] has become popular. Tocrisolve™ is a registered preparation consisting in a vehicle designed for lipophyllic compounds such as cannabinoids and the vanilloid agonists. Tocrisolve™ is made up of soya oil in a proportion of 1:4 with water and emulsified with the F 68 pluronic copolymer. It allows dissolving WIN 55212-2 up to a concentration of 2%. On the other hand, it does not require the use of ethanol to promote its dissolution and has demonstrated sustained ocular penetration of the WIN 55212-2 agonists dissolved therein after topical application.[68]

Δ9-THC, dissolved in mineral oil, demonstrated a reduction of intraocular pressure higher than that obtained by pilocarpine (52%) and with a longer effect.[38,68] This hypotensive effect has been reproduced in various studies with different cannabinoids (table 2).

However, not all studies presented to date agree in granting said beneficial effects to cannabinoids in the field of glaucoma. Some studies have questioned the effectiveness of the CB1 cannabinoid receptor agonists because this hypotensive effect is not reproduced after the application of WIN-55212-2 having high affinity with CB1 receptors.[69]

Side effects of cannabinoids after ocular systemic/topical application

The ocular side effects after topical or systemic administration of cannabinoids are very few. The acute side effects include tachycardia, orthostatic hypotension, euphoria and conjunctival hyperemia.[70] The longer-term side effects include respiratory, hormonal and neurological side effects. Smoking marijuana has been associated to emphysematous pulmonary changes caused by marijuana combustion products, as occurs with many cannabinoids, or due to the release of carcinogens and other volatile substances that occur in greater concentrations than with tobacco smoking.[66] The topical application of Δ9-THC, CBN or CBD has been associated to midriasis, conjunctival hyperemia, chemosis, cases of severe corneal opacification and neurotoxicity.[70] Other ocular side effects associated with systemic administration pathways of cannabinoids are diminished tear production, diplopia, accommodation alterations, photophobia, nistagmus and blepharospasms.[7,10,66,70]

Conclusions

From the control of intraocular pressure up to correct trophism of ganglion retina sends, the endogenous endocannabinoid system plays an important role in ocular physiology.[75-78] An increased knowledge of receptors and pathways through which cannabinoids can exert their multiple ophthalmological effects will prompt us to consider these drugs as therapeutic tools for medical treatment of glaucoma.

Numerous experimental and clinical studies have endorsed the role of cannabinoids as ocular hypertensors, regulating the main risk factor in the development of glaucoma. Although the exact mechanism is not known yet, it seems that the activation of CB1 receptors, widely expressed in the trabecular mesh and the non-pigmented epithelium of the ciliary body, are mainly responsible for the ocular hypertensive effects. In addition, through the CB1 and CB 2 receptors as well as the non-CB1/CB 2 receptors, cannabinoids have also demonstrated protective effects over the retina ganglion cells.

The use of new solvents such as Tocrisolve™ and 2-hydroxypropil-β-cyclodextrine have allowed an appropriate dissolution of cannabinoids and the preparation of solutions for ocular topical application. Even though the results obtained to date give rise to hope for their application in the field of glaucoma, more studies are necessary to determine with greater precision their security, together with clinical trials to assess the usefulness of these compounds for treating glaucoma in our daily practice.

Conflict of interest

None of the authors have declared any conflict of interest.

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