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Review

Is there a rational basis for cannabinoids research and development in ocular pain therapy? A systematic review of preclinical evidence

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ABSTRACT

Background: Purpose of the present systematic review is to investigate preclinical evidence in favor of the working hypothesis of efficacy of cannabinoids in ocular pain treatment.
Methods: Literature search includes the most relevant repositories for medical scientific literature from inception until November, 24 2021. Data collection and selection of retrieved records adhere to PRISMA criteria.
Results: In agreement with a priori established protocol the search retrieved 2471 records leaving 479 results after duplicates removal. Eleven records result from title and abstract screening to meet the inclusion criteria; only 4 results are eligible for inclusion in the qualitative synthesis impeding meta-analysis. The qualitative analysis highlights the antinociceptive and anti-inflammatory efficacy of Δ8-tetrahydrocannabinol, cannabidiol and its derivative HU-308 and of new racemic CB1 allosteric ligand GAT211 and its enantiomers GAT228 and GAT229. Moreover, CB2R agonists RO6871304 and RO6871085 and CB2R ligand HU910 provide evidence of anti-inflammatory efficacy. CB2 agonist HU308 reduces of 241% uveitis-induced leukocyte adhesion and changes lipidome profile. Methodological and design issues raise concern of risk of bias and the amount of studies is too small for generalization. Furthermore, the ocular pain model used can resemble only inflammatory but not neuropathic pain.
Conclusions: The role of the endocannabinoid system in ocular pain is underinvestigated, since only two studies assessing the effects of cannabinoid receptors modulators on pain behavior and other two on pain-related inflammatory processes are found. Preclinical studies investigating the efficacy of cannabinoids in ocular inflammatory and neuropathic pain models are needed to pave the way for clinical translation.

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1. Introduction

1.1. Rationale

Ocular pain can be difficult to diagnose and it can present a multifaceted nature, nociceptive and neuropathic, due to the complex pain signaling network in the eye. For instance, any causes of ocular surface damage, e.g. trauma and inflammation (uveitis, photorefractive surgery or invasive procedures) or infection (keratitis or endophthalmitis) can induce ocular nociceptive pain [2]; on the other hand, ocular neuropathic pain is due to injury of the somatosensory nociceptive system and it is often chronic, reducing the quality of life, ranging from the dry eye disease (DED) and the chronic dry eye-like pain (DELP) to the centralized oculofacial neuropathic pain syndrome [44]. In particular, keratitis, e.g. surgical, post-herpetic and neurotrophic keratitis, has an estimated prevalence of 1.6/10,000 people [12] and DED has a prevalence ranging from 5% to 60% increasing with age [58], affecting more than 1.68 million men and 3.23 million women, with a remarkable burden on ability to perform daily living activities and on health utility, originating measure of quality-adjusted life-years (QALYs) loss, comparable to psoriasis and class III/IV angina [13]. Also, the economic burden of DED is noteworthy both for its direct medical and indirect costs in terms of lost work time and reduced productivity [39]. Therefore, pharmacological treatments to gain QALYs are urgently needed. DED has been associated to depressive mood [70] and it is one, but not the only, cause of ocular neuropathic pain [34] that can be due to keratoneuralgia as well or it can be the evolution of an infectious process, e.g. post-herpetic neuralgia [18]. The human cornea and conjunctiva are highly innervated. The nociceptive system involved in ocular pain transmission consists of peripheral nociceptors located in the nasociliary branch of the ophthalmic division of trigeminal ganglion sensitive to mechanical, thermal and chemical stimuli [44]. In particular, the axons project to second-order ocular neurons or interneurons in the trigeminal subnucleus interpolaris/caudalis transition region and in the caudalis/upper cervical cord junction [2]. The second-order projections terminate in regions of the pain ascending pathway, i.e. periaqueductal gray, hypothalamus, amygdala, and prefrontal cortex, but also to superior salivatory and facial motor nuclei responsible for lachrymation and eyeblink, respectively [2]. Furthermore, the presence on nociceptors of the transient receptor potential TRPM8 confers responsiveness to evaporation (evaporative hyperalgesia), cooling and hyperosmotic tears [15]: the latter are responsible for DELP [44]. This explains the inflammatory and neuropathic nature of ocular pain conditions as DED. In fact, tear deficiency, occurring in immune mediated diseases as Sjögren's Syndrome [21], induces inflammation [23] and consequent nociceptors sensitization [14]. A neurosensory dysfunction can be a component of DED as well [14]. Also, systemic neuropathies, e.g. diabetes [21], and ocular surgery, e.g. laser in situ keratomileusis (LASIK) [16,22], can induce ocular chronic pain. Moreover, ocular neuropathic pain due to injury of peripheral corneal nerves is often underestimated due to the lack of differential diagnosis with DED [34]. Finally, phantom eye pain is described in the frame of phantom eye syndrome, underestimated complication of eye amputation [28].

1.2. The challenge of topical ocular pain treatment

A fundamental issue is the therapy of ocular pain, since a definite cure does not exist. In fact, several topically administered drugs with different mechanisms of action can be used [34]: therapies aimed at improving lubrication; non-steroidal anti-inflammatory drugs (NSAIDs, nepafenac or ketorolac) and steroids (e.g. hydrocortisone) to reduce inflammation; antibiotics (doxycycline or azithromycin) and immunomodulators (cyclosporine) in case of meibomian gland dysfunction; tacrolimus for tear stability. As for ocular inflammatory pain there is not a specific therapy for neuropathic corneal pain, whose treatment consists in topical antineurodegenerative and antinflammatory treatments (autologous serum tears and corticosteroids, the latter, apart from hydrocortisone [11], endowed with the risk of increased intraocular pressure, glaucoma, cataract and secondary infections for their immunosuppressive action) and in the systemic administration of tricyclic antidepressants (nortriptyline, desipramine) and serotonin-noradrenaline reuptake inhibitors (SSRI, duloxetine) or anticonvulsants (carbamazepine, gabapentin, pregabalin), naltrexone, tramadol or the sodium channel blocker mexiletine [8]. The endocannabinoid system has proven to be an important target in multiple neuropathic pain models [27] as for the reduction of chemotherapy-induced allodynia [36] and it has recently been implicated in ocular pain. In fact, anadamide (AEA), 2-arachidonoylglycerol (2-AG) and the cannabinoid receptors have been identified in the human cornea and ocular tissues [4,60,61]. Their role in ocular pain can include both immunomodulatory/antiinflammatory and pain modulatory action [19]. For instance, in the animal model of ocular inflammation and uveitis induced by intraocular administration of lipopolysaccharide, the activation of the CB2 receptor has been associated to reduced leukocyte adhesion and pro-inflammatory mediators, showing superiority to dexamethasone, prednisolone and nepafenac [67]. Incidentally, the activation of the CB1 receptor can reduce chemical excitation by mustard oil of corneal afferent fibers of the trigeminal interpolaris/caudalis and subnucleus caudalis/upper cervical cord areas and CO_2-induced magnitude of single unit activity recorded at level of the trigeminal interpolaris/caudalis transition, while morphine fails in these outcomes [3]. Therefore, the aim of this systematic review is to assess the efficacy of the modulation of the endocannabinoid system through CB1 and CB2 receptor agoizts, antagonists and allosteric modulators in animal models of ocular inflammatory and neuropathic pain. To the best of our knowledge, this is the first systematic review of preclinical studies on the analgesic effects of modulators of the endocannabinoid system in nociceptive and neuropathic pain models.

2. Methods

2.1. Protocol

the protocol for the search to address the latter participants, interventions, comparisons, outcomes, and study design (PICOS) question follows the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) criteria [24,32]. It is prespecified and the retrieved results are double-checked by two independent researchers and any conflicts are resolved by a third author. Since the purpose of this study is to evaluate the body of preclinical evidence in favor of clinical translation of endocannabinoid modulators for ocular pain treatment without an already reported outcome of clear human relevance, the present protocol was not eligible for registration in the International prospective register of systematic reviews PROSPERO.

2.2. Inclusion criteria

these include in vivo studies assessing the effects of agonists, antagonists and allosteric modulators of the CB1 and CB2 receptors administered topically on the nociceptive response induced by ocular inflammatory and neuropathic, acute and chronic, pain models. Also, studies assessing the effects of agonists, antagonists and allosteric modulators of the CB1 and CB2 receptors administered topically on apparently pain-related outcomes, e.g. anti-inflammatory effects, are included. Studies performed on both male and female rodents are considered eligible for inclusion, taking into account that possible differences in ocular pain, i.e. in the model of lacrimal gland excision [30], may occur according to gender, which can also influence the effect of tetrahydrocannabinol on tearing along with CB1 mRNA and protein levels in lacrimal gland [65]. Both nociceptive behavior assessment and apparently pain-related molecular outcomes, e.g. leukocyte adhesion and neutrophil migration, are investigated. Studies need to be published in English and no filters about publication date are applied.

2.3. Exclusion criteria

in vivo studies not respecting the animal welfare are established not to be eligible for inclusion. Clinical or in vitro studies, narrative or systematic reviews and meta-analysis, abstracts, congress communications, poster and proceedings, editorials, encyclopedia and book chapters and studies not available in full text and not published in English are excluded.

2.3.1. Information sources

the most relevant databases for medical scientific literature are searched, including PubMed/MEDLINE, Scopus, Web of Science, ScienceDirect and Mendeley from their date of inception to the date of last search, that is November, 24th 2021. It is not possible to perform the literature search also on EMBASE, being the latter not freely/institutionally available.

2.4. Search strategy

The following search terms, modifications and combinations are used: ocular pain, ocular pain syndrome, chronic ocular pain, corneal pain, corneal inflammatory pain, corneal neuropathic pain, ocular inflammatory pain, ocular neuropathic pain, ocular acute pain, ocular chronic pain, corneal acute pain, corneal chronic pain, corneal neuralgia, keratoneuralgia, corneal neuropathic disease, dry eye disease, dry eye-like pain, oculofacial neuropathic pain syndrome, corneal allodynia, corneal hyperalgesia, cannabinoids, endocannabinoid agonists, endocannabinoid antagonists, endocannabinoid (allosteric) modulators, CB1 (R) agonists, CB1(R) antagonists, CB2(R) agonists, CB2(R) antagonists, CB1(R) (allosteric) modulators, CB2(R) (allosteric) modulators, antinociceptive activity, anti(i)nflammatory activity, antiallodynic activity, antihyperalgesic activity.

2.5. Data collection, selection and analysis process

the retrieved results and the duplicates are double-checked by two independent researchers to minimize the risk to exclude relevant records and any conflicts to reach consensus are resolved by a third author. The search and the eligibility evaluation is performed independently by two authors and the references list of the articles is examined to extend and refine the search. A complete consensus in the whole collection and selection process was reached without relevant conflicts. The risk of bias and the quality of the studies are assessed by two independent researchers using the Systematic Review Center for Laboratory Animal Experimentation (SYRCLE's) risk of bias (RoB) tool [17] and the Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies (CAMARADES) checklist for study quality [26]. A systematic synthesis of the results is carried out following the Cochrane Consumers and Communication Review Group guidelines [46].

3. Results

3.1. Process of collection and selection of the studies

The first screening assesses title and abstract after the duplicate records elimination and, then, the full texts are evaluated for eligibility. The search retrieved 2471 records from databases without any additional results from other searches. The records were subjected to duplicates removal leaving 479 results. Title and abstract screening to carry out an initial exclusion of clinical trials, narrative or systematic reviews and meta-analysis, in vitro studies, abstracts and congress communications, poster and proceedings, editorials, encyclopedia and book chapters and in vivo studies not meeting the inclusion criteria, provided 11 records all published in English and available in full text. The screening of the latter full texts led to the inclusion of 4 records in the qualitative analysis. In particular, two studies met the pain assessment outcome and two the apparently pain-related anti-inflammatory outcomes.

3.2. Studies excluded after full text analysis

the studies by De Petrocellis et al. [7] and Moriello et al. [33] are excluded because, notwithstanding the use of the eye-wiping assay as pain model, no cannabinoids are studied. The study by Murataeva et al. (2019) is conducted on male and female mice of C57BL/6Jand CD1 strain, with a part on CB1 and CB2 knock-out, and corneal injury is induced through mechanical debridement, similarly to Yang et al. [74], but the study of migration is performed using cultured bovine corneal epithelial cells, therefore the study is not eligible for inclusion. Furthermore, although testing JWH-250 and JWH-073 that are two synthetic cannabinoid agonists with nanomolar affinity at CB1 and CB2 receptors, the paper of Ossato et al. [37] is eliminated because the pain models are not ocular. The study by Spyridakos [59] and collaborators is excluded since it applies a model of retinal excitotoxicity. Also, the study conducted by Yang and colleagues [74] does not focus on pain even though studying the endocannabinoid signaling and using a model of eye alkali burn. In this study the selective CB1 agonist WIN55,212–2 improves wound healing reducing tissue contraction indicative of myofibroblast transdifferentiation, but the effect of the latter agonist on monocyte and neutrophil stromal infiltration is not reported thus not making the study eligible for inclusion. Finally, the research of Thayer et al. [65] cannot be included because it studies the effect of the endocannabinoid system on tearing, important in DED, but without investigating ocular pain or anti-inflammatory pain-related outcomes. The studies resulted eligible for inclusion in the qualitative analysis are the two studies by Thapa and coworkers [63,64] and the studies by Porter [38] and by Toguri [68] and colleagues.

The collection and selection process is illustrated in the PRISMA flow diagram (Fig. 1).

3.3. Qualitative synthesis and RoB assessment

the data obtained from the studies included in the qualitative analysis are grouped and analyzed according to Cochrane Consumers and Communication Review Group guidelines [46]. The number of studies for both the nociceptive outcome ($n = 2$) and the pain-related outcomes ($n = 2$) is too small ($n < 5$) impeding accurate comparison and quantitative meta-analysis. The studies included in the qualitative analysis for the pain assessment outcomes are the two ones by Thapa and coworkers [63,64], thus research comes from the same group and this is mirrored in the methodology. The study conducted by Thapa et al. in 2018 [64] assesses the antinociceptive and antiinflammatory actions of the topical administration of phytocannabinoids Δ8-tetrahydrocannabinol, cannabidiol and its derivative HU-308 in a model of corneal hyperalgesia, obtained through application of capsaicin on the cornea previously subjected to chemical cauterization with silver nitrate, in wild-type (WT) and CB2R knockout ($CB2R^{-/-}$) mice. A dose-response study is performed and the concentrations of 1% Δ8-tetrahydrocannabinol, 5% cannabidiol and 1.5% HU-308 result effective in significantly reducing the pain score consisting in the sum of the number of blinks, squints and eye wipes in WT mice in response to capsaicin. Moreover, the latter cannabinoids significantly reduce neutrophil number in the cornea in comparison with vehicle (soybean oil). On the other hand, only 1% Δ8-tetrahydrocannabinol and 5% cannabidiol show antinociceptive and antiinflammatory properties in $CB2R^{-/-}$mice. The intraperitoneal (i.p.) pretreatment with the CB1R antagonist AM251 inhibits the antinociceptive and antiinflammatory actions of Δ8-tetrahydrocannabinol, but not of cannabidiol. The i.p. treatment with the 5-HT1A receptor antagonist WAY100635 blocks the corneal antinociceptive and anti-inflammatory activity of cannabidiol. Therefore, the actions of Δ8-tetrahydrocannabinol are linked to CB1R, the activity of HU-308

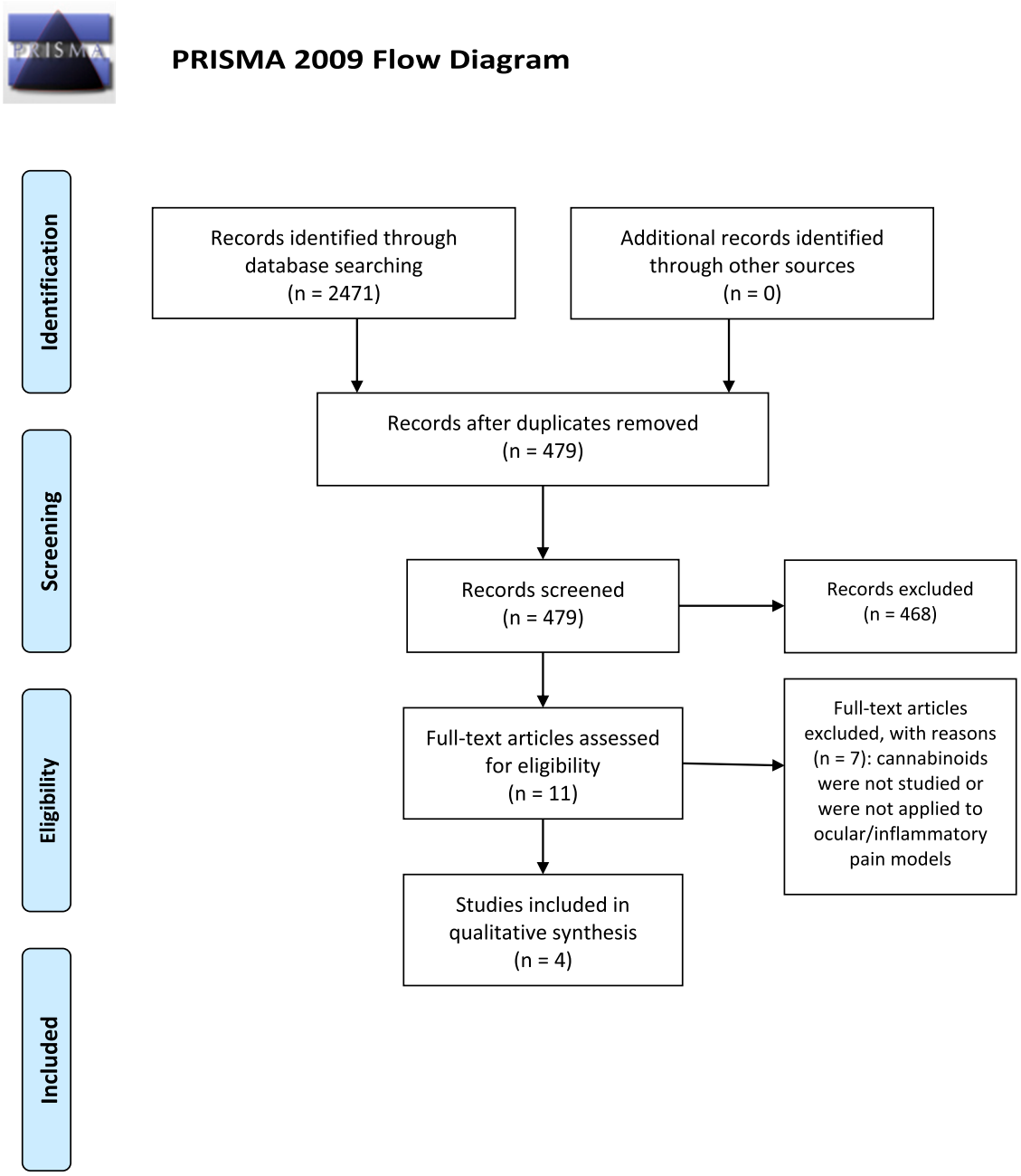

Fig. 1. Search process and selection of the relevant records eligible to be included in qualitative analysis based on the Preferred Reporting Items for Systematic reviews and Meta-Analyses (PRISMA) criteria [32].

depends on CB2R and the properties of cannabidiol are independent on both endocannabinoid receptors but are dependent on 5-HT1A receptors. Also, in the study published by Thapa et al. in 2020 [63] the corneal hyperalgesia model is obtained through the application of capsaicin on the cornea previously subjected to chemical cauterization with silver nitrate. In the latter study [63] the new racemic CB1 allosteric ligand GAT211 and its enantiomers GAT228 and GAT229 are investigated for their antinociceptive and antiinflammatory properties, also in combination with the orthosteric CB1 agonist Δ8-tetrahydrocannabinol. Only GAT228 provides analgesic efficacy, while 0.5% GAT229 or 1% GAT211 demonstrate antinociceptive properties when in combination with Δ8-tetrahydrocannabinol, 0.4% solution being subthreshold. GAT228 and GAT229 are effective also in $CB2R^{-/-}$ mice, but pretreatment with i.p. AM251 blocks their effect. Therefore, the GAT229 and GAT228 antinociceptive properties are underlined by CB1R, but not CB2R, dependent mechanisms. The neutrophil infiltration is reduced by 2% GAT228 and by 0.4% Δ8-tetrahydrocannabinol alone and in the percentage of 0.2% in combination with 0.5% GAT229. The studies included in qualitative analysis for pain-related outcomes of leukocyte-neutrophil activity and immune cells infiltration are those by Porter et al. [38] and by Toguri et al. [68]. In the study of Porter et al. [38] the topical CB2R agonists RO6871304 and RO6871085 and the CB2R inverse agonist RO6851228 are studied and characterized in their binding and pharmacokinetic profiles emphasizing their lipophilicity and capability to cross membranes, blood–brain barrier and blood–retina barrier (apart from RO6871304). RO6871304 and RO6871085 and the CB2R ligand HU910 significantly reduce the leukocyte-endothelial interactions due to uveitis induced by an intravitreal injection of LPS (endotoxin-induced uveitis) in WT mice, with RO6871304 proving the highest effectiveness. Consistently, the CB2R

inverse agonist RO6851228 increases leukocyte-endothelial adhesion. The research by Toguri et al. [68] does not focus on pain, despite studying the CB2 agonist HU308 in a model of uveitis. In particular, HU308 significantly reduces the leukocyte adhesion and neutrophil recruitment induced in the BALB/c wild-type mice and only leukocyte adhesion in CB2 knock-out mice, suggesting the involvement of CB2R in HU308 anti-inflammatory action.

The main features of the studies according to the PICOS question are illustrated in Table 1.

All the possible forms of bias were assessed. Selection bias is evaluated both in terms of sequence generation (allocation sequence able to produce comparable groups) and of baseline characteristics (comparable and not adjusted for confounders in the analysis). The study design of the study of by Thapa et al. [63] parallels the study of 2018 [64]. Both methodologies are correct. The genotyping of the animals is accurately performed and reported in each study. Male BALB/c as WT animals and $CB2R^{-/-}$ mice are used, but, interestingly, in 2020 study by Thapa et al. the animal baseline characteristics of age are matched and the adherence to the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Vision Research has been reported. The same animals are used also by Porter et al. [38], reporting that the two groups are age-matched and the exact genotyping. BALB/c wild-type and CB2 knock-out mice are used in the study of Toguri et al. [68] reporting the exact genotyping. In the latter study by Toguri et al. [68] all leukocyte adhesion studies are reported to be conducted during the same time of day and mice are age and sex matched and from multiple litters. Moreover, the lack of allocation concealment during the enrollment contributes to originate selection bias and of random housing and randomization during the study gives rise to performance bias. No mention about random housing is reported in any of the studies. Performance and detection biases also consist in absent blinding of investigators during the study and of outcome assessors, respectively. Behavioral experiments are not planned at specific time during the day and random assignment or blinded assessment are not reported in the study of 2018 [64]. However, blinded data analysis of coded mice is carried out in the study of 2018 by Thapa et al. [64]. On the contrary, in the study of 2020 off-line analysis is reported to have been conducted by an experimenter blinded to the treatments [63]. In the study by Porter et al., [38] and Toguri et al., [68] individual animals in each treatment group are coded and experiment analysis is blinded. Animals eventually excluded from outcome assessment represent attrition bias and reports free of selective outcome reporting prevent

Table 1
Main characteristics of the studies eligible for inclusion in qualitative analysis.

Study	Cannabinoids	Route of administration	Comparator or vehicle	Animals	Ocular pain model	Analgesic and apparently pain-related anti-inflammatory outcome and sample size	Results
[64]	Δ8-tetrahydrocannabinol, cannabidiol and its derivative HU-308	Topical	Soybean oil (vehicle); CB1R antagonist AM251	Male BALB/c (wild-type) and CB2R knockout mice ($CB2R^{-/-}$)	Corneal hyperalgesia	Pain score consisting in the sum of the number of blinks, squints, and eye wipes in response to capsaicin after corneal chemical cauterization. N = 5–12 per group	1% Δ8-tetrahydrocannabinol, 5% cannabidiol and 1.5% HU-308 reduce pain score and neutrophils infiltration in wild type mice. Only 1% Δ8-tetrahydrocannabinol and 5% cannabidiol are effective in $CB2R^{-/-}$. The antinociceptive and antiinflammatory properties of Δ8-tetrahydrocannabinol, but not of cannabidiol, are prevented from the intraperitoneal pretreatment with the CB1R antagonist AM251 and the i.p. treatment with the 5-HT1A receptor antagonist WAY100635 blocks the corneal antinociceptive and anti-inflammatory activity of cannabidiol
[63]	CB1 allosteric ligands GAT211, GAT228, and GAT229 and Δ8-tetrahydrocannabinol	Topical	Soybean oil (vehicle); CB1R antagonist AM251	Male BALB/c (wild-type) and CB2R knockout mice ($CB2R^{-/-}$)	Corneal hyperalgesia	Number of blinks and eye wipes as described in Thapa et al. [64]	GAT228 demonstrates antinociceptive properties, while those of 0.5% GAT229 or 1% GAT211 occur only in combination with 0.4%. Δ8-tetrahydrocannabinol. GAT228 and GAT229 are effective also in $CB2R^{-/-}$mice, but pretreatment with i.p. AM251 blocks their analgesic effect. 2% GAT228 and by 0.4% Δ8-tetrahydrocannabinol alone and in the percentage of 0.2% in combination with 0.5% GAT229 reduce neutrophil infiltration.
[38]	CB2R agonizts RO6871304 and RO6871085, CB2R inverse agonist RO6851228 and CB2R ligand HU910	Topical	Tocrisolve	Male BALB/c wild-type and age-matched CB2R-/- knock-out mice	Endotoxin-Induced Uveitis (EIU)	Leukocyte adhesion and neutrophil migration during EIU	Topical 1.5% w/v RO6871304, or RO6871085 significantly attenuates EIU-induced leukocyte-endothelial interactions compared to vehicle ($p < 0.05$). RO6851228 increases iridal leukocyte adhesion
[68]	CB2 agonist HU308 (4-[4-(1,1-dimethylheptyl)-2,6-dimethox-yphenyl]-6,6-dimethylbicyclo[3.1.1]hept-2-ene-2-methanol)	Topical (1.5% w/v)	Tocrisolve	Mal BALB/c wild-type and age-matched CB2 knock-out mice	EIU	Leukocyte adhesion during EIU	HU308 reduces of 241% the leukocyte adhesion in wild-type mice and changes the lipidome profile

reporting bias. Other sources of bias can be represented by the lack of evidence of induced pain using the selected behavioral outcome measure before the administration of the interventions and the examination (i.e., sham procedure), a clear description of methods with the number of animals used, attention to circadian regulation for behavioral studies, use of the same observer for behavioral tests, use of control and positive and negative control drugs, sample size calculation, statement of conflict of interest, statement of compliance with animal welfare regulations and attention to ethics. In all the studies included in qualitative analysis the animal welfare is respected in agreement with the established eligibility criteria. All the studies included follow the Canadian Council for Animal Care guidelines, apart from submitting the protocol to the Dalhousie University Committee on Laboratory Animals. The total number of animals per group is stated and specified according to the single experiment in the studies by Thapa et al. [64] and Porter [38] and Toguri [68] and coworkers. However, the number of animals per treatment group differs and no reason is discussed. In this case [63] the sample size is reported for each experiment, but also the number of animals per treatment group differs and no reason is discussed. Finally, the compliance with the guidelines for Animal Research: Reporting In Vivo Experiments (ARRIVE) [40] are not reported in any study by Thapa, but the latter are stated in the study by Porter [38] and Toguri [68] and coworkers. Sham/control procedure is performed in the first study. The existence of any conflicts is disclosed in both studies by Thapa et al. A summary of the signaling questions used to assign a judgment of low, high or unclear risk of bias to each item mentioned in the RoB assessment tool is provided in Table 2.

4. Discussion and conclusions

The role of the endocannabinoid system in pain modulation is widely investigated, but ocular pain is still an under evaluated field. The need to study cannabinoids in models of ocular diseases and of DED, has recently emerged since they could be more effective and safer than traditional immunosuppressive therapeutics [1]. In fact, there is evidence suggesting the endocannabinoids, mainly acting at CB2R, as key players in inflammation [66,67,69,73]. Therefore, the investigation of the efficacy of the cannabinoids in ocular inflammatory diseases and inflammatory pain is mandatory. Furthermore, the role of the latter neurotransmitter system in the descending, pain inhibitory, pathway [54] apart from the anti-inflammatory action, supports the need to study the pharmacological modulation of the endocannabinoid system in neuropathic pain. This issue is of great importance since alternative pain treatments could increase time in treatment before the loss of efficacy of analgesics and reduces the dosages of systemically administered painkillers that could be endowed with serious adverse reactions. This is even more important for ocular neuropathic pain that is treated with systemic antidepressants or anticonvulsants, being a topical treatment still lacking. However, the preclinical research in this field has not provided a strong rationale in favor of the translation for the clinical use of cannabinoids in ocular pain. In fact, the results of the present systematic review prove a scarce scientific production. Thus, 2471 records were retrieved and only 4 of these investigate the antinociceptive properties of cannabinoids in ocular models. The two studies by Thapa and coworkers highlight the antinociceptive and antinflammatory efficacy of Δ8-tetrahydrocannabinol, cannabidiol and its derivative HU-308 and of the new racemic CB1 allosteric ligand GAT211 and its enantiomers GAT228 and GAT229. The study by Porter and coworkers highlights that the CB2R agonists RO6871304 and RO6871085 and the CB2R ligand HU910 show anti-inflammatory effects, as the CB2 agonist HU308, also able to affect the lipidome profile, investigated by Toguri and colleagues. Therefore, the strength of the present systematic review is that it demonstrates the poor and not methodologically flawless research supporting the role of the endocannabinoid system in ocular pain, having retrieved only two studies assessing the effects of cannabinoid receptors modulators on pain

Table 2
Risk of Bias (RoB) assessment of the studies eligible for inclusion in qualitative analysis. "Yes" indicates low risk of bias; "no" indicates high risk of bias; and "unclear" indicates an unclear risk of bias.

Studies	Signaling questions									
	Was the allocation sequence adequately generated and applied?	*Were the groups similar at baseline or were they adjusted for confounders in the analysis?*	*Was the allocation to the different groups adequately concealed during?*	*Were the animals randomly housed during the experiment?*	*Were the caregivers and/or investigators blinded from knowledge which intervention each animal received during the experiment?*	*Were animals selected at random for outcome assessment?*	*Was the outcome assessor blinded?*	*Were incomplete outcome data adequately addressed?*	*Are reports of the study free of selective outcome reporting?*	*Was the study apparently free of other problems that could result in high risk of bias?*
[64]	Unclear	Unclear	Unclear	No	Yes	No	No	Unclear	Unclear	Yes
[63]	Unclear	Yes	Unclear	No	Yes	No	No	Unclear	Unclear	Yes
Porte et al. (2018)	Unclear	Yes	Unclear	No	Yes	No	No	Unclear	Unclear	Yes
[68]	Unclear	Yes	Unclear	No	Yes	No	No	Unclear	Unclear	Yes

behavior and other two on pain-related inflammatory processes. For the latter reason, this work highlights the need to deepen the study of the cannabinoid modulators resulted from the systematic analysis in ocular pain models relevant to clinic to build the rational basis for translation. Apart from the paucity of studies found that does not allow meta-analyses and generalization of the results, an important limitation of the study is that it was not possible to perform the literature search also on EMBASE since it is not freely/institutionally available. Although a good methodological design and the interesting pharmacodynamic and pharmacokinetic characterization of the latter compounds, further studies are needed to generalize these findings for several reasons. The number of studies is too small to allow a meta-analysis that can determine if the analgesic treatment of ocular pain with cannabinoids is favored respect to vehicle. Moreover, the lack of allocation concealment, random housing and blinding of observers as well as the missing consideration of circadian rhythm for behavioral tests are among other factors that originate some concern in term of RoB. The currently approved cannabinoids are dronabinol and nabilone for chemotherapy-associated nausea and vomiting and dronabinol also for human immunodeficiency virus (HIV)-associated anorexia, therefore their use in pain treatment is still off-label [71]. In Italy, the first drug approved based on cannabinoids is Sativex (oral spray formulation containing nabiximol, including Δ9-tetrahydrocannabinol and cannabidiol) for the treatment of spasticity associated to multiple sclerosis (MS) and there is evidence for efficacy in MS induced central pain [41]. This could represent the basis for the development of a topical formulation for the treatment of ocular pain. To this aim, the issues raised by ocular topical delivery formulations need to be addressed and overcome, in particular poor ocular bioavailability of solutions, suspensions and ointments, representing the most common ocular formulations [31]. In fact, drainage from the pre-corneal site by blinking reflex or lachrymation is very fast compelling to frequent administrations worsening the possible associated ocular irritation and blurring vision [31]. Furthermore, the physicochemical features of the active principles must be taken into account to allow the development of an effective corneal topical formulation that could require semisolid nanoparticles/nanoemulsion gels [31]. Ongoing researches show that novel cannabinoids for pain and inflammation treatment can exploit the 2-pyridone ring scaffold [10]. Another important problem raised by this search is the lack of proper ocular neuropathic pain models. The application of topical capsaicin on the corneal tissue after chemical cauterization is the model used by the studies included in the qualitative synthesis and it can represent only inflammatory pain as the alkali (e.g. NaOH) burn corneal pain models [6]. In fact, although the tissue injury can affect also nervous terminations with partial corneal denervation [6] and the sensitization induced by inflammatory processes can lead to neuropathic pain, the typical markers as the upregulation of the L-type voltage gated Ca^{2+} channels subunit α2δ-1 [25] are identified in the trigeminal ganglion in these models. Notwithstanding the presence of markers of brainstem synaptic plasticity, the same occurs for the DED models consisting in excision of the extra orbital lachrymal gland and of the Harderian gland [9]. Due to the biomarker induced the best model of ocular surface inflammation with implication for allodynia and neuropathic pain development for preclinical studies may be represented by the use of benzalkonium chloride [5]. However, apart from ocular alkali burn [72], photokeratitis [62] and induction of DED [20], an ocular neuropathic pain model consisting in the ligation [57] of the ciliary branches innervating the cornea has been described [29]. The problem of chronic ocular pain deserves stronger research effort also because it is associated with brain-related changes involved in the impairment of memory and of cognitive functions [44], leading to underdetection and inappropriate treatment of pain [49,52,56], even more so during the pandemic [51]. Furthermore, neurodegenerative diseases, e.g. Alzheimer's and Parkinson's disease, and retinal ganglion cells alterations are associated and share molecular pathways [35,45]. Therefore, preclinical research needs to provide future studies on both inflammatory and neuropathic ocular pain models characterizing the pharmacological modulation of the sensitization at level of the trigeminal ganglion operated by the cannabinoids. This process has been followed for essential oils, among which bergamot has proven strong evidence of analgesia in inflammatory and neuropathic pain models, thus being eligible for translation into clinic [42,43,47,48,50,53,55] and showing potential also for ocular pain treatment in topical formulation. This can form the rational basis for the translation of the use of cannabinoids for the treatment of ocular pain into clinic.

Conflict of interest

The authors declare no conflict of interest.

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No funding has been received for this trial. This is a non-profit study, in which no form of remuneration has been foreseen for study participants and for the chief investigator and all the staff involved.

CRediT authorship contribution statement

Conceptualization: D.S., G.B., M.T.C., P.T., T.S., S.S., S.M., C.N.; Data curation, Formal analysis, Methodology: D.S., L.R, K.H., F.G., S.B., G.A. G. All authors have read and approved the final manuscript.

Declarations of interest

None.

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