Combination therapy and co-delivery strategies to optimize treatment of posterior segment neurodegenerative diseases

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Neurodegenerative diseases affecting the posterior segment of the eye are one of the major causes of irreversible blindness worldwide. The pathogenesis of these retinal pathologies is characterized by a multifactorial etiology, involving the complex interaction of different apoptotic mechanisms, suggesting that effective treatments will require a multimodal approach. Thus, combination therapy based on the potential synergistic activities of drugs with different mechanisms of action is currently receiving considerable attention. Here, we summarize several kinds of strategy for the co-administration of different drugs to the posterior segment of the eye, highlighting those that involve co-delivery from multiloaded drug delivery systems.

Introduction

Neurodegenerative diseases affecting the posterior segment of the eye are one of the major causes of irreversible blindness worldwide. These pathologies include glaucoma, age-related macular degeneration (AMD), diabetic retinopathy (DR), and diabetic macular edema (DME), among others [1]. By 2020, the number of people affected by AMD is expected to rise to 8.8 million, by glaucoma to 4.5 million, and by DR to 3.2 million [1]. The damage occurring in these neurodegenerative pathologies is not confined to the primary insulted neurons, because the following secondary injuries also affect neighboring neurons [2]. The pathogenesis of retinal diseases is characterized by a multifactorial etiology that involves the complex interaction of metabolic, functional, genetic, and environmental factors [3]. The apoptotic cascade of neuronal cells includes several mechanisms, such as excitotoxicity, protein misfolding, mitochondrial dysfunction, oxidative stress, inflammation, and neurotrophin deprivation [4–7]. Moreover, the events involving retinal cell death can interact and compound, suggesting that an effective treatment requires a multimodal approach.

Until now, most used monotherapies have been insufficiently effective at preventing neurodegenerative progression. Attributed to the potential synergistic activities of drugs with different mechanisms of action, combination therapy is currently receiving considerable attention. This is the case for several therapeutic combinations based on anti-inflammatory drugs and anti-vascular endothelial growth factor (VEGF) molecules, aim at increasing the control over inflammatory pathways and vascular disorders [8]. Inflammation has a main role in ischemic retinopathies, such as DR, AMD, and other vascular disorders of the retina, such as retinal vein occlusion (RVO) [9,10]. VEGF has also a main role in these diseases, not only as a mediating cytokine in inflammation, but also as a disrupter of the blood–retinal barrier leading to an increase in vascular permeability [10,11]. Likewise, fixed-combination therapies of topical antiglaucomatous drugs are currently used in clinical practice. Nevertheless, despite the clinical efficacy obtained by simultaneous administration of drugs administered topically, there are some limitations when the target site is located in the posterior segment, such as short half-life of drugs, *in vivo* pharmacokinetics, or intraocular distribution of the drugs, which can compromise this therapeutic strategy [12].

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Intraocular drug delivery systems (IODDSs) could address the aforementioned crucial issues for optimal therapy. Thus, the use of IODDs based on implants, nano-, and microsystems to vitreoretinal disorders treatment has been widely investigated. In addition, given the main characteristics of neurodegenerative diseases affecting the optic nerve and the retina (chronicity, multifactorial etiology, and progressive degeneration) novel therapeutic strategies based on combined therapy and the co-delivery of drugs from IODDS are also being developed.

Here, we review different strategies for the co-administration of different drugs to the posterior segment of the eye, which are under either clinical use or investigation. We also discuss recent combination therapy-based strategies involving drug delivery systems and future possibilities in this field (Table 1, Fig. 1).

Combination therapy using free drugs

Combination therapy through the simultaneous administration of multiple therapeutic agents has emerged as a crucial approach to achieve enhanced activity through synergistic effects [13–15].

Simultaneous administration of active substances has demonstrated to have benefits compared with monotherapy. As cited earlier, several fixed combinations are now used to reduce intraocular pressure (IOP) in patients with glaucoma. This is the case for prostaglandins (bimatoprost, latanoprost, or travoprost) with timolol maleate (TM) [16–18], which act through different mechanisms. Whereas prostaglandins analogs increase the uveoscleral outflow drainage mediated by Prostaglandin F receptor (FP) activation, TM reduces aqueous humor production by adrenergic receptor blockage. As a result of this combination therapy, an additive effect is obtained [19]. In fact, because of the benefits observed in IOP reduction, this therapeutic strategy has been extended to a triple combination of drugs (prostaglandin analogs combined with a brinzolamide/timolol maleate fixed combination) [13]. Brinzolamide is a specific carbonic anhydrase (CA) inhibitor that lowers IOP by reducing the rate of aqueous humor formation [20].

However, these antiglaucoma fixed-combination therapies require at least twice-daily dosing, which often leads to decreased patient adherence. Novel fixed-dose combination products, such as Roclatan™, simplify dosing regimens by providing comparable efficacy with a once-daily eye drop regimen. Roclatan™ is a fixed-dose combination product for IOP reduction comprising latanoprost alongside a Rho kinase inhibitor (netarsudil) [21]. Inhibitors of Rho kinase have emerged as a new class of IOP-lowering drugs capable of increasing outflow through the trabecular meshwork [22].

Combination therapy has been extended to other ocular disorders, such as for DME. The pathogenesis of DME is complex and includes multiple mechanisms, the most notable of which are angiogenesis and inflammation [23]. Although intravitreal injections of anti-VEGF are still first-line treatment, not all patients respond optimally to anti-VEGF therapy [24]. In these cases, according to the data from several clinical trials, combinations of anti-VEGF with corticosteroids or non-steroidal anti-inflammatory drugs (NSAID) are emerging as promising therapeutic alternatives [25–27]. In addition, other novel options are under investigation to explore the benefit of combination therapy. An oral curcumin formulation [DIABEC (2 tablets/day); curcuma solid extract with 20% of curcuminoids] in combination with dexamethasone intravitreal injection (700 μg) is currently in a clinical trial for its impacts on morphological retinal characteristics and retreatment times in patients with DME (NCT03598205) [28]. Dexamethasone is a synthetic glucocorticoid with potent anti-inflammatory activity via inhibition of inflammatory cells and suppression of the expression of inflammatory mediators and immunosuppressive effects. Curcumin has been described as an antioxidant, anti-inflammatory, and antitumor agent [29]. In ophthalmic disorders, it has been found to attenuate several mechanisms implicated in disease pathogenesis, including mitochondrial-mediated oxidative stress, inflammatory responses via PPAR-γ agonist activity, downregulation of cyclooxygenase-2 (COX-2; an enzyme involved in the inflammatory response) and inducible nitric oxide synthase (iNOS; a key enzyme generating nitric oxide), and antiangiogenic activity via modulation of the VEGF/VEGFR/K-ras pathway [30–32].

In AMD, a combination of aflibercept injection (Eylea®, 2 mg) and a topical NSAID (bromfenac) (Yellox® 0.09%) resulted in higher efficacy in terms of visual acuity improvement compared with a single anti-VEGF therapy (Eylea®, 2 mg) [33–35] According to the authors, the inclusion of the NSAID results in a decrease in the number of injections required, leading to economic benefits. In addition, a combinaton therapy using a platelet-derived growth factor (PDGF) antagonist [E10030: Fovista (pegpleranib)] and ranibizumab (Lucentis®) has been tested in patients (Phase IIb clinical studies; NCT01089517) [36]. The authors hypothesized that VEGF and PDGF inhibition would cause neovascular regression. In this study, patients were randomized in three groups receiving E10030 (0.3 mg) with 0.5 mg of ranibizumab; E10030 (1.5 mg) with 0.5 mg of ranibizumab; and 0.5 mg of ranibizumab as monotherapy. All groups received monthly intravitreal injections with a total administration of six doses (over 24 weeks). The combined therapy was well tolerated and patients experienced a considerable improvement in visual acuity as well as neovascular regression. However, according to two pivotal Phase III clinical trials (OPH1002 and OPH1003), the addition of the anti-PDGF therapy to a monthly ranibizumab regimen did not result in benefits compared with anti-VEGF monotherapy for wet AMD after 1 year of treatment. Likewise, a Phase III clinical trial (OPH1004) demonstrated that Fovista (anti-PDGF therapy) in combination with Eylea (aflibercept) or Avastin (bevacizumab) did not result in any clinically meaningful visual benefit compared with anti-VEGF monotherapy for the treatment of wet AMD after 12 months of treatment. Although the safety profile of both the combination and monotherapy remained unchanged from previous trials, ocular adverse events were more frequently reported in the combination treatment. These were mainly related to the injection procedure, which was performed as two separate intravitreal injections, which could contribute to treatment failure [37]. These data have put into perspective how important it is to develop other potential combination targets.

Several studies have evaluated combination therapies based on other therapeutic modalities, such as photodynamic therapy (PDT) with anti-VEGF and anti-inflammatory agents. A pilot study of the triple therapy was carried out in choroidal neovascularization (CNV) secondary to AMD [38]. Intravitreal dexamethasone (800 μg) and bevacizumab (1.5 mg) were administered 16 h after verteporfin PDT. The triple combination therapy addresses three main targets of CNV

TABLE 1

Summary of combination therapies based on fixed-combination of free drugs, implant + free drug and co-delivery from ocular drug delivery systems (hydrogels, contact lenses, nanoparticles and microparticles) for the treatment of retinal diseases and other intraocular pathologies[a]

Combination	Disease	Mechanism of action	*In vitro*/animal (healthy or animal model)/clinical trial	Outcomes	Refs
Free drugs					
Bimatoprost + timolol maleate (BTFC); latanoprost + timolol maleate (LTFC); travoprost + timolol maleate (TTFC)	Glaucoma	IOP-reducing prostaglandins (uveoscleral flow increase) + β-adrenoceptor antagonist (decrease aqueous humor production)	Clinical trial: prospective, observer-masked, randomized patients with primary open-angle glaucoma: Group BTFC ($n = 18$); Group LTFC ($n = 14$); Group TTFC ($n = 18$)	No significant differences in diurnal or nocturnal IOP variation between the three groups ($P > 0.05$).	[18]
Prostaglandin therapy (latanoprost/ tavoprost/bimatoprost/ tafluprost) + brinzolamide + timolol maleate	Glaucoma	IOP-reducing prostaglandin analog (uveoscleral flow increase) + noncompetitive reversible CA inhibitor + β-adrenoceptor antagonist (decreases aqueous humor production)	Clinical trial: prospective, interventional, single-arm, open-label study; 38 patients with open-angle glaucoma or ocular hypertension (NCT01263444)	At week 12, 70% of patients achieved IOP ≤ 18 mmHg (mean IOP at week 12 17.2 ± 4.1 mmHg versus 23.1 ± 3.0 mmHg at baseline)	[13]
Roclatan™ latanoprost + netarsudil	Glaucoma	IOP-reducing prostaglandin (uveoscleral flow increase) + Rho kinase inhibitor	Clinical trial: double-masked, randomized, parallel comparison study; 298 patients with open-angle glaucoma or OHT	Fixed-dose combination met criterion for statistical superiority relative to both latanoprost and netarsudil ($P < 0.0001$), providing additional IOP lowering of 1.9 and 2.6 mmHg, respectively.	[21]
Dexamethasone + DIABEC (curcumin tablets)	DME	Anti-inflammatory (corticosteroid) + antioxidant, anti-inflammatory, and antitumor agent	Clinical trial: NCT03598205	Recruiting	
Aflibercept (IVA) + bromfenac (B)	AMD	Antiangiogenic agent + NSAID	Clinical trial: prospective, randomized; Group IVA ($n = 27$); Group IVA/B ($n = 27$)	Visual acuity improved in IVA/B group	[33]
E10030 + ranibizumab (IVR)	AMD	PDGF antagonist + anti-angiogenic agent	Clinical trial: NCT01089517 Phase III clinical trials (OPH1002 and OPH1003)	Combined therapy showed improvement in visual acuity and neovascular regression Addition of anti-PDGF therapy to monthly ranibizumab regimen did not result in benefit compared with anti-VEGF monotherapy	[36]
Verteporfin photodynamic therapy + bevacizumab + dexamethasone	CNV secondary to AMD	Photodynamic therapy + anti-angiogenic agent + anti-inflammatory (corticosteroid)	Clinical trial: prospective, NONCOMPARATIVE, interventional case series; 104 patients with all types of CNV secondary to AMD	Mean increase in visual acuity by 1.8 lines ($P < 0.01$); mean decrease in retinal thickness 182 μm ($P < 0.01$)	[38]
Zimura™ + Lucentis	AMD	Complement factor C5 inhibitor + anti-angiogenic agent	Clinical trial: NCT03362190	Completed; no results posted	
Propranolol + bevacizumab (IVB)	AMD	β-blocker + anti-angiogenic agent	Clinical trial: NCT03609307	Recruiting	
TRC105 + ranibizumab (IVR)	AMD		Clinical trial: NCT03211234	Recruiting	
Implant + free drug					
Dexamethasone (Ozurdex®) + aflibercept (IVA)	DME	Anti-inflammatory (corticosteroid) + anti-angiogenic agent	Observational and retrospective study; Patients with DME: Group IVA ($n = 15$); Group IVA/Ozurdex® ($n = 15$)	BCVA increased with aflibercept (70.8 ± 4.1 to 83.5 ± 2.7 letters) and with sequential treatment (from 75.6 ± 2.7 to 86.5 ± 2.5) (P5 0.551); CMT decreased from 411 ± 26.1 to 288.1 ± 10.5 with aflibercept and from 411.4 ± 24.3 to 260.8 ± 17.9 in the sequential treatment group	[55]

TABLE 1 (*Continued*)

Combination	Disease	Mechanism of action	*In vitro*/animal (healthy or animal model)/clinical trial	Outcomes	Refs
Dexamethasone (Ozurdex®) + bevacizumab	ME	Anti-inflammatory (corticosteroid) + anti-angiogenic agent	Retrospective, nonrandomized, open-label case series study; 20 patients with ME	BCVA improved significantly from 0.758 ± 0.42 logarithm of the minimum angle of resolution (logMAR) (at baseline) to 0.51 ± 0.33 logMAR at 1 month and to 0.5 ± 0.34 logMAR at 2 months ($P \leq 0.03$)	[46]
	ME	Anti-inflammatory (corticosteroid) + antiangiogenic agent	Clinical trial: interventional, prospective, nonrandomized, noncomparative open-label, single-center investigation case series; 24 eyes of patients with ME;BRVO ($n = 15$); CRVO ($n = 9$)	Mean BCVA gained 0.313 ± 0.26 (85.3% of final gain) and 0.367 ± 0.34 at week 1 and month 6, respectively; percentage of patients who gained $\geq$2 lines 52% at Week 1 and 68% at Month 6; mean macular thickness reduced by 350.9 mm at Week 1 and the maximum treatment effect was seen at Month 2 (379.1 mm).	[47]
	RVO	Anti-inflammatory (corticosteroid) + antiangiogenic agent	Clinical trial: prospective, nonrandomized, open-label, single-center investigation, interventional case series; 34 patients: $n = 12$ RVO and $n = 22$ BRVO	Mean visual acuity improved from initially 11 letters to a maximum of 25 letters; macular thickness decreased with combination treatment	[49]
Dexamethasone (Ozurdex®) + bevacizumab (IVB)	DME	Anti-inflammatory (corticosteroid) + anti-angiogenic agent	Clinical trial: prospective, single-masked, randomized, single-center investigation, controlled trial; patients with DME: Group IVB ($n = 19$); Group IVB/ Ozurdex® ($n = 21$)	Mean reduction in central subfield thickness greater in combination group (−45 μm versus-30 μm, difference5 69 Mm, 95% confidence interval5 9-129; $P = 0.03$). More patients in the combination group had central subfield thickness <250 mm	[53]
	DME	Anti-inflammatory (corticosteroid) + antiangiogenic agent	Clinical trial: prospective, nonrandomized study; patients with DME: Group IVB ($n = 20$); Group IVB/ Ozurdex® ($n = 15$)	Dexamethasone implanted in 15 eyes (42.8%) unresponsive to IVB at 18 weeks. Statistically significant improvements observed in BCVA (at postoperative 4 and 12 weeks) and CFT (at postoperative 4, 12 and 24 weeks) in IVB/Ozurdex® group	[54]
	RVO	Anti-inflammatory (corticosteroid) + anti-angiogenic agent	Clinical trial: prospective, consecutive, nonrandomized case series; Patients with RVO: Group Ozurdex® ($n = 22$ CRVO and $n = 16$ BRVO); Group Ozurdex®/IVB ($n = 14$ CRVO and $n = 12$ BRVO)	BCVA improved in Ozurdex® group [by 6.6 (±1.7) letters in CRVO and 7.8 (±2.9) in BRVO] and in Ozurdex®/IVB group [by 9.8 (±1.0) versus 9.4 (±2.1) letters]; significant difference only seen between CRVO patients in Ozurdex® group and 2 at 12 months ($P < 0.05$)	[48]
	BRVO	Anti-inflammatory (corticosteroid) + anti-angiogenic agent	Retrospective case series; patients with BRVO: Group IVB ($n = 26$); Group IVB/ Ozurdex® ($n = 20$)	BCVA in first month significantly better in IVB/Ozurdex® group (logMAR IVB/ Ozurdex® group 0.21 ± 0.26 versus IVB group 0.39 ± 0.30, $P = 0.038$) and the 1-month CMT was thinner (IVB/ Ozurdex® group 270.0 ± 62.0 μm versus IVB group 338.9 ± 122.6 μm, $P = 0.028$)	[50] [b]

TABLE 1 (*Continued*)

Combination	Disease	Mechanism of action	*In vitro*/animal (healthy or animal model)/clinical trial	Outcomes	Refs
Dexamethasone (Ozurdex®) + ranibizumab (IVR)	Macular edema (ME)	Anti-inflammatory (corticosteroid) + antiangiogenic agent	Clinical trial: retrospective cohort study; Patients with ME: Group IVR ($n = 13$); Group Ozurdex®/IVR ($n = 13$)	Significant improvements in BCVA ($P = 0.04$) and CMT ($P < 0.01$) were achieved in the Ozurdex®/IVR group	[45] [c]
	Subfoveal CNV secondary to AMD	Anti-inflammatory (corticosteroid) + anti-angiogenic agent	Clinical trial (NCT01162746): randomized and controlled; patients with CNV secondary to AMD: group IVR (n = 20); group Ozurdex®/IVR (n = 20)	First retreatment was delayed significantly ($P = 0.004$) compared with IVR group; visual acuity changed from 62 letters at baseline to 67 at month 12 in IVR group and remained stable at 68 letters in Ozurdex®/IVR group ($P = 0.68$) Macular sensitivity changed from 6.95 dB to 7.01 dB in IVR group and from 7.24 dB to 7.12 dB in Ozurdex®/IVR group($P = 0.4$)	[51]
Hydrogels					
Brimonidine + TM	Glaucoma	IOP reducing	*In vitro* drug uptake studies (human corneal epithelial cells); *Ex vivo* drug uptake studies (bovine eyes)	DH increased PBS solubility of brimonidine and sustained *in vitro* release of both drugs over 56–72 h; compared to PBS-drug solutions, DH significantly increased human corneal epithelial cells uptake and bovine corneal transport for both drugs.	[59]
Contact lenses					
Timolol maleate + dorzolamide + vitamin E	Glaucoma	IOP reducing	Beagle dogs (primary open-angle glaucoma)	CL-based therapy reduced IOP with lower drug dose compared with eye drops; might significantly improve compliance	[63]
Nanoparticles					
Resveratrol + quercetin	Glaucoma	IOP reducing	Normotensive rabbits	Resveratrol and quercetin-loaded PEG-modified chitosan NPs showed sustained and enhanced reduction of IOP (5.5 ± 0.5 mmHg) in normotensive rabbits	[64]
Microspheres					
GDNF + vitamin E	Glaucoma	Neuroprotection	Rodent model of chronic OHT (Morrison rat model of glaucoma)	Intravitreal injection of GDNF/vitamin E PLGA microspheres in experimental animal model of glaucoma significantly increased RGC survival compared with GDNF, vitamin E, or blank microspheres ($P < 0.01$); effect present for at least 11 weeks	[65]
GDNF + melatonin	Retinal degeneration	Neuroprotection	Rhodopsin-knockout mice	Intravitreal injection of GDNF or GDNF/melatonin-loaded MSs led to partial functional and structural rescue of photoreceptors compared with blank microspheres or vehicle	[69]
Dexamethasone + melatonin + coenzyme Q10	Glaucoma	Neuroprotection	Rodent model of chronic OHT (Morrison rat model of glaucoma)	21 days after OHT induction, DMQ-MSs showed significantly neuroprotective effect on retinal ganglion cells	[70]

[a] Abbreviations: B, bromfenac; BTFC, fixed-combination of bimatoprost + TM; IVA, intravitreal injection of aflibercept; IVB, intravitreal injection of bevacizumab; IVR, intravitreal injection of ranibizumab; LTFC, fixed-combination of latanoprost + TM; TTFC, fixed-combination of travoprost + TM.
[b] Kangdong Sacred Heart Hospital, Seoul, Korea (KANGDONG 2016-09-010).
[c] Institutional Review Board at Show Chwan Memorial Hospital (Registration No. 1060502).

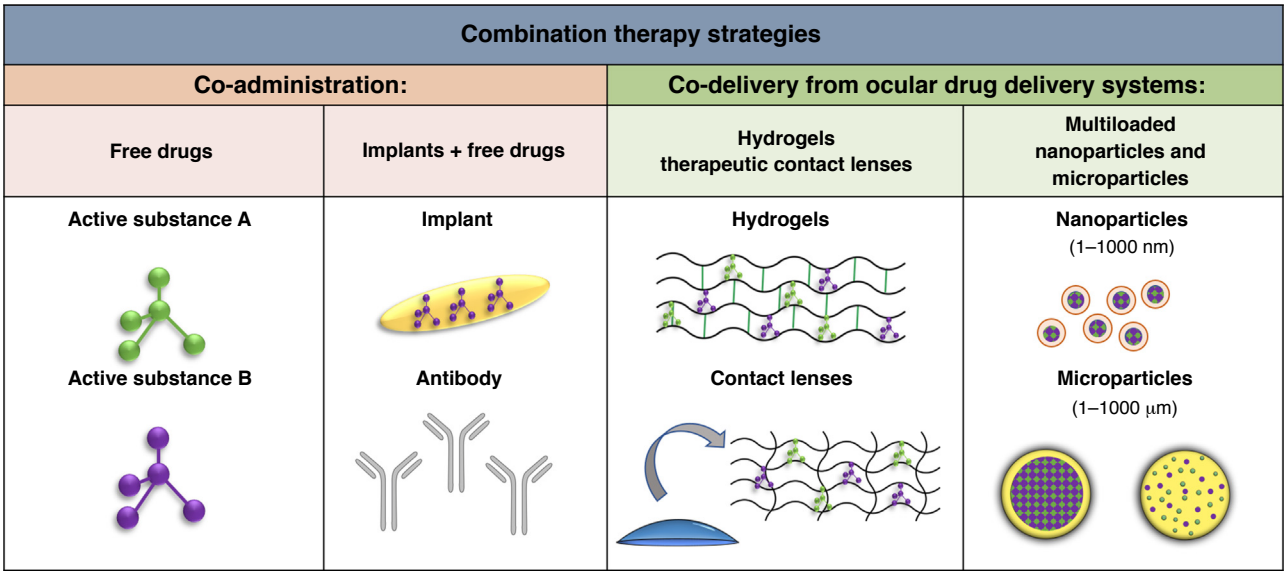

FIGURE 1

Combination therapies based on a fixed combination of free drugs, implant + free drug, and co-delivery from ocular drug delivery systems (hydrogels, contact lenses, nanoparticles, and microparticles) for the treatment of retinal diseases and other intraocular pathologies.

development: the CNV itself, VEGF expression (which promotes CNV growth), and inflammation (which exacerbates the disease process) [38]. In this study, 104 patients received a single triple-therapy cycle (five patients received a second triple treatment because of remaining CNV activity and, in 18 patients, the triple therapy was complemented by an additional intravitreal injection of bevacizumab). In most patients, no serious adverse events were observed, resulting in significant ($P < 0.01$) and sustained visual acuity improvement after one cycle of treatment.

Complement dysregulation induces the pathogenesis of AMD given that, upon activation of the complement system, activation fragments (such as C3a and C5a) can amplify and exacerbate inflammation and tissue injury. Moreover, the presence of C5 components has been described in drusen and RPE cells overlying or directly adjacent to the drusen [39]. Thus, a combination of a complement factor C5 inhibitor (ZIMURA™) plus an anti-VEGF agent ranibizumab (Lucentis®; 0.5 mg) has been studied (NCT03362190) [40]. The benefit of administering beta-blockers to modulate the vasoproliferative retinal process has also been hypothesized, and a combination of intravitreal bevacizumab (anti-VEGF agent) and propranolol injection has been assayed (NCT03609307; currently recruiting) [41,42]. In addition, endoglin/CD105 has been highlighted as a potential therapeutic vascular target [43]. This component is overexpressed in vascular endothelial cells in soft tissues undergoing angiogenesis, and levels of CD105 correlate with endothelial cell proliferation. A combination of an antiendoglin antibody (TRC105) plus an anti-VEGF agent ranibizumab (Lucentis®) is under evaluation (NCT03211234) [44].

Combination of implants and free drugs

Despite the synergy achieved by several fixed combination therapies through the simultaneous administration of free drugs, the outcomes for neurodegenerative ocular diseases treatments are still unsatisfactory in terms of a short drug half-life, repeated intravitreal administrations, or undesired toxicity effects. Recently, emerging combination therapies involving ocular implants and free drugs have been investigated to provide more selective targeting along with the sustained release of molecules at the target site.

Although intravitreal anti-VEGF therapy has been shown to prevent the loss of vision in most neovascular diseases, results can only be assured if successive injections are administered. Given that repeated injections are a burden on the patient, attempts are being made to overcome this issue by combining available treatment options.

A combination therapy based on the dexamethasone implant Ozurdex® {a biodegradable polymer [poly(lactic-co-glycolic acid)] matrix containing 700 μg of dexamethasone able to provide 6-months release} and monthly ranibizumab (2.3 mg) injections for macular edema was described by Lin *et al.* [45]. Patients who received concurrent Ozurdex® and ranibizumab injections from 2012 to 2016, with a follow-up period of at least 3 months, were studied. The efficacy of the treatment was demonstrated through the significant improvement in visual acuity and central macular thickness in the study group (13 patients) compared with controls (13 patients), who only received intravitreal injections of the anti-VEGF [45].

Simultaneous therapy combining intravitreal Ozurdex® with intravitreal injection of bevacizumab (Avastin®; 1.25 mg) has been investigated in short-term treatment. At the end of a 60-day study, a significant decrease in macular thickness was registered in patients with macular edema secondary to DR and RVO treated with this concomitant therapy, with an acceptable safety profile of the treatment regimen [46]. Nagpal *et al.* showed that the combination therapy of the dexamethasone implant (Ozurdex®, 700 μg) and a single bevacizumab (1.25 mg) injection given simultaneously, was both safe and synergistic, providing significantly sustained

visual recovery and decreased macular edema in 24 patients with RVO 6 months after the start of the study [47]. Likewise, Mayer *et al.* [48] compared Ozurdex® as monotherapy with a combination of three intravitreal injections of bevacizumab (1.25 mg) also including Ozurdex® to treat RVO-associated macular edema in a 12-month treatment. Intravitreal injections of bevacizumab were administrated monthly and a dexamethasone implant (Ozurdex®) was put in place at week 16. Overall, 26 patients received the combined therapy and 38 patients the monotherapy (Ozurdex®). According to the authors, the combination did not prolong the recurrence period. In patients with central retinal vein occlusion (CRVO), the combination showed better functional improvement. Conversely, according to the authors, the monotherapy (Ozurdex®) showed reduced retinal thickness (measured by optical coherence tomography) in patients with brain retinal vein occlusion (BRVO). Further studies that include a longer period of time would be needed to verify the benefits of dexamethasone monotherapy (Ozurdex®) in patients with BRVO. The previously mentioned combination of dexamethasone implant (Ozurdex®; 700 μg) and bevacizumab (1.25 mg) in 34 patients with RVO showed a synergistic effect that resulted in an increase in visual acuity and a delay of repeated injections, compared with Ozurdex® or Avastin® alone, in a 6-month study [49].

In addition, the comparison of the synergic effects of intravitreal bevacizumab (Avastin®; 1.25 mg) and dexamethasone implant (Ozurdex®; 700 μg) for the treatment of macula edema associated with BRVO with a single administration of intravitreal bevacizumab was studied by Moon *et al.* in a retrospectively review of medical records over a period of 15 months [50]. Intravitreal bevacizumab was administrated to 26 patients and the cited combination was received by 20 patients. After the first administration, all patients were examined once per month, receiving an intravitreal injection of bevacizumab in the absence of improvement in, or intensification of, central macular thickness. Although significant differences were not found with regard to final visual and anatomical results, the cited study suggested that the use of dexamethasone implant together with an intravitreal injection of bevacizumab enhanced the recovery of patients during the first month in contrast to the administration of monotherapy (bevacizumab). However, more patients throughout a longer period of time would be necessary to validate these findings.

A dexamethasone implant (Ozurdex®) was also combined with ranibizumab (Lucentis®; 0.5 mg) delaying retreatment with the anti-VEGF in patients with persistent and/or recurrent AMD. This study [a randomized and controlled clinical trial (NCT01162746)] was performed for 12 months in 40 patients with choroidal neovascularization secondary to AMD. Patients were randomized in two groups receiving either an initial dose of ranibizumab or the antibody plus an injection of the dexamethasone implant [51]. The combination was based on the anti-inflammatory, antiangiogenic, and antiedematous effects of dexamethasone and a reduction of neovascularization in response to ranibizumab [51,52].

Given that laser photocoagulation has shown insufficient improvement in visual acuity in DME, there has been a growing interest in other treatment methods, including fixed-combination strategies combining intravitreal antibodies targeting VEGF and sustained-release intravitreal steroid implants. A 12-month study involving 30 patients (40 eyes) determined that a dexamethasone intravitreal implant (Ozurdex®; 700 μg) combined with bevacizumab (1.25 mg) provided greater benefit than bevacizumab monotherapy in patients with DME with incomplete response to multiple anti-VEGF injections [53]. Combination therapy eyes received intravitreal bevacizumab at baseline and a dexamethasone implant at month 1, 5, and 9, whereas monotherapy eyes received bevacizumab (monthly) if indicated. According to the authors, the dexamethasone implant plus bevacizumab significantly improved visual acuity and macular morphology in eyes with refractory diabetic macular edema. Likewise, Güler *et al.* demonstrated significant improvement in the visual acuity in nonresponder patients with DME treated with a fixed treatment protocol combining anti-VEGF therapy (bevacizumab; 2.5 mg) and the dexamethasone implant (Ozurdex®; 700 Mg) [54]. Sequential treatment in DME, starting with the implant followed by anti-VEGF treatment (aflibercept; 2 mg), has also been reported as a promising alternative that significant decreased macular thickness compared with aflibercept monotherapy [55].

Co-delivery from ocular drug delivery systems

It is well known that the bioavailability of topically administered drugs is compromised by factors that limit their access to the posterior segment of the eye and that <5% of the dose reaches the intraocular tissues [12]. Systemically administered drugs cannot achieve therapeutic concentrations in the posterior segment of the eye because of the blood–aqueous and blood–retinal barriers [56]. Thus, most drugs are administered directly into the eye by intravitreal injection if the target site is located in the back of the eye. Despite the advantage of local administration, repeated intravitreal injections are required with increasing potential risks of complications, such as cataract formation, retinal detachment, or endophthalmitis, among others [57].

By contrast, the many individual medications that patients must self-administer increase treatment complexity and can reduce therapeutic compliance [13]. The development of drug delivery systems capable of providing effective therapeutic drug concentrations, while minimizing adverse effects and improving patient adherence, would be beneficial in clinical practice. Thus, efficient treatment of ocular diseases can be achieved as a result of the proper use of drug delivery technologies based on emerging pharmaceutical approaches. There are different kinds of drug delivery technology designed to serve as ODDS. These include, among others, hydrogels, therapeutic contact lenses (CLs), nanosystems, and microsystems.

Hydrogels: therapeutic contact lenses

Hydrogels are 3D polymeric networks capable of the controlled release of active substances trapped inside [58]. Hydrogels have received considerable attention because their chemical and physical versatility. This versatility can be exploited to attain sustained-release therapies. Topical administration of hydrogels is one of the most frequent drug delivery routes for the treatment of ocular pathologies affecting the ocular surface or when the drug has to reach the anterior segment. Hydrogels can also be engineered as implants that can be injected intracamerally for anterior segment therapies or intravitreally for posterior segment therapies.

A new combined topical formulation comprising a dendrimer hydrogel (DH) formed of polyamidoamine (PAMAM), polyethyl-

ene glycol (PEG)-acrylate chains and loaded with a combination of brimonidine and TM (0.1% and 0.5% w/v, respectively) was developed for the treatment of glaucoma [59]. The solubility of poorly water-soluble brimonidine was enhanced as a result of the hydrophobic core of the PAMAM dendrimers. This dendrimer hydrogel resulted in higher human corneal epithelial cell uptake compared with brimonidine and a timolol maleate eye drop solution (control). The *in vitro* release of both drugs was also prolonged, in contrast to eye drop formulations (PBS-drug solutions). Moreover, the hydrogel significantly increased bovine corneal transport for both drugs compared with an eye drop formulation with the same concentration of brimonidine and timolol.

The potential interest in sustained drug delivery to the eye via a CL system is understandable. CLs are able to extend drug release towards the post-lens tear fluid if their composition and inner architecture are fitted to the features of the drug molecules [60]. However, there are different drawbacks, including regulatory aspects. CLs could be considered as a subclass of combination products and, depending on the main mode of action regarding CL function or the drug effect, different guidelines must be considered. In addition, ophthalmic diseases, such as glaucoma, mainly occur in older patients, who are less likely to wear and apply CLs. Also, the burst effect associated with soaked CLs can limit their application. Novel polymers and elaboration techniques have led to a renaissance of the use of CLs for therapeutically purposes [61].

Drug-eluting CLs can be prepared using CLs already used for correction of refractive errors via embedding or interacting with the polymers of the network. In addition, high compliance is required by patients with refractive error-correcting or -designed CLs. *Ad hoc*-designed CLs involve the elaboration of copolymers with functional capacities able to interact with target molecules. For example, a bioinspired approach was used to develop epalrestat-eluting CLs. The presence of the bioactive monomer monomethacryloxypropyl-sym-polydimethylsiloxane hydroxypropyl terminated (MCS-MC12), capable of interacting with aldose reductase and epalrestat, regulated the release rate of the drug. Aldose reductase is an enzyme that reduces glucose into sorbitol in hyperglycemia conditions leading to retinopathy and cataracts. Epalrestat is an aldose reductase inhibitor and MCS-MC12 interacts with the active site of the enzyme, thereby inhibiting it [62].

Hsu and colleagues embedded TM (12.75 mg/ml) and dorzolamide (20 mg/ml) in therapeutic CLs with vitamin E to control the release of both drugs. The combination of TM and the carbonic anhydrase inhibitor (CA inhibitor) dorzolamide resulted in more of a decrease in IOP compared with monotherapies in glaucoma. In addition, vitamin E enables better controlled release of the drugs longer periods of time even when the CL is removed because of the deposition of lipidic complexes of tocopherol in the ocular surface [63].

Multiloaded nano- and microparticles

Multiloaded micro- and nanoscale carriers have emerged as novel strategies for enhanced combinatorial treatments. Considerable efforts are being devoted to developing co-delivery systems incorporating several drugs into a single carrier system and subsequently releasing the multiple payloads at the desired target site in a controlled manner. Natesan *et al.* tested PEG-modified chitosan nanoparticles (NPs) loaded with two natural anti-oxidants: resveratrol and quercetin, in rabbits. Developed NPs showed sustained release of both active compounds and synergic effects on reducing IOP [64]. According to the authors, previous clinical studies reported that oxidative stress and inflammation are associated with trabecular meshwork cell dysfunction increasing the aqueous outflow resistance and, thus, IOP.

By contrast, Checa-Casalengua *et al.* used PLGA microspheres (MSs) of glial cell line-derived neurotrophic factor (GDNF) and vitamin E (Vit E), to effectively protect retinal ganglion cells (RGC) in a rodent model of ocular hypertension [65]. GDNF is a prosurvival neurotrophin with recognized potential for the treatment of a variety of neurodegenerative diseases [66]. From a pharmacological point of view, the antioxidant properties of Vit E might help to reduce the oxidative stress associated with glaucomatous optic neuropathy [67]. Moreover, a pharmacokinetic study carried out with this formulation provided sustained release of the neurotrophic factor in a controlled fashion for up to 6 months after a single intravitreal injection in rabbits [68]. In the same way, a recently published study reported the ability of a PLGA-based slow-release formulation of MSs loaded with GDNF in combination with an antioxidant (melatonin) to rescue photoreceptors in a mouse model of retinal degeneration (rho$^{-/-}$ mice) [69]. In addition, a novel multiloaded microparticulate formulation incorporating three recognized neuroprotective agents [dexamethasone (anti-inflammatory), melatonin (antioxidant), and coenzyme Q10 (an essential cofactor in mitochondrial bioenergetics)] have been shown to lead to the functional and structural rescue of RGC in a well-established rodent model of chronic OHT after 1 month of administration. Thus, this formulation is presented as a novel therapeutic approach in the management of retinal neurodegeneration conditions, such as glaucoma [70].

Concluding remarks and perspectives

Here, we presented a summary of the recent combination treatments that have been developed and evaluated for chronic neurodegenerative pathologies affecting the posterior segment. Combination therapy takes advantage of the strengths of each monotherapy and their different mechanisms of action to achieve good treatment outcomes with few repeated treatments. Promising synergistic effects have been achieved through the combination therapy of free drugs. Nevertheless, the optimization of the synergy could be compromised by different aspects, including short *in vivo* half-lives, insufficient drug delivery in the target site, or adverse effects. This is the case of fixed-combination therapies based on free drugs currently used in clinical practice requiring intense follow-up and frequent intravitreal injections. Accordingly, various therapeutic strategies involving carrier systems have been developed for the co-administration of several active substances in a controlled manner. Initially, these approaches were based on drug delivery systems and aided the intravitreal administration of free drugs, enhancing both drug distribution and the stability of the molecules and releasing the active agents at the target sites in a sustained manner. Currently, the development of multiloaded carriers could result in long-acting injectable drug formulations capable of delivering different drugs over longer time periods, offering an excellent alternative to multiple administrations. Moreover, with this novel therapeutic strategy, a smaller amount of biomaterial is injected because the different drugs are included in the same microcarrier.

The development of treatments involving combinations of drugs is a promising approach towards combating complex or multifactorial disorders. However, adverse effects and tolerability of combination therapies can be a concern. Adverse effects can include a higher risk of adverse reactions or increased overall costs. To minimize these aspects, there are several factors that can determine whether drug combinations modify the risk of adverse reactions, mainly whether a pathway targeted by a drug also contains off-targets that would cause adverse effects.

Under a European Regulatory standpoint, an individual fixed combination will only be considered acceptable if the proposed combination is based on valid therapeutic principles. It is necessary to assess the potential advantages (an improvement of the benefit:risk assessment or a simplification of therapy) in the clinic against possible disadvantages (e.g., cumulative toxicity) for each fixed combination product and for each dose of the fixed combination product. Given that the possibility of interactions between the substances always has to be considered, pharmacodynamic and pharmacokinetic data should be provided to the relevant regulatory authority. Safety studies in animals should be performed with the active substances of the fixed combination in the proportion present in the product. Regarding therapeutic aspects, confirmatory clinical trials are necessary to prove efficacy, preferably by parallel group comparisons in which the fixed combination is compared with its individual substances. Comparative clinical studies of the fixed combination versus reference treatment might be necessary. Combination products are marketed under a single marketing authorization [71,72].

According to the U.S. Food and Drugs Administration (FDA), to determine whether an investigational application is needed for a combination product, the regulatory requirements for an investigational new drug application (IND) or an investigational device exemption application (IDE) should be considered. Typically, an IND is submitted if the combination product has a drug or biological primary mode of action (PMOA) or as an IDE if the combination product has a device PMOA. In fact, combination products are typically marketed under a single marketing authorization type associated with the constituent part that provides the PMOA for the combination product [i.e., a new drug application (NDA) or abbreviated new drug application (ANDA) if it has a drug PMOA, a biologic license application (BLA) if it has a biologic PMOA, or a premarket approval application (PMA) *de novo* certification, or premarket notification]. Clinical studies as the primary support for the safety and effectiveness of a combination product for a proposed indication should also be provided [73,74].

Overall, combination therapies have been increasingly used either to improve compliance or to benefit from the added effects of the two medicinal products given simultaneously. Such improvements in the field of ocular drug delivery research based on combinatorial treatments could bring about novel methods for the better management of ocular diseases.

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