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Overcoming Barriers: Controlled-Release Systems as Vectors, the Posterior Segment of the Eye Approach as a Model

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Authors' contributions

This work was carried out in collaboration between both authors. Authors JRV and LJRV designed the study, managed the literature searches and wrote the article equally. Both authors read and approved the final manuscript.

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ABSTRACT

Background: A successful therapy requires drug access to the target site. This is particularly complicated if the target is located in a well-protected location. Furthermore, if a chronic disease must be treated, the desirable system should be able to control drug release, maintain therapeutic concentrations during the necessary period and prolong administration needs.
Objective: To serve such purposes, the use of controlled-release systems as vectors has been suggested. This might be relevant, among other regions, in the posterior segment of the eye, a place where access is difficult due to different barriers.
Results: To achieve access, multiple strategies have been attempted. Dendrimers, microparticles and liposomes have been designed with varying success. The lack of clinical trials is an issue that must still be addressed.
Conclusion: These approaches are only the tip of the iceberg in terms of what pharmaceutical technology will develop over the next decades.

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1. INTRODUCTION

A targeted therapy is one that has been developed to affect a specific target, such as an enzyme or a receptor. Targeted therapies can either block or increase the function of their target to treat a particular disease. This is subsequent to the drug's general basis of action. For example, in the treatment of ocular diseases, spironolactone, a steroidal competitive mineralocorticoid receptor antagonist drug, can prevent receptor overactivation by endogenous hormones or increased levels of glucocorticoids, contributing to retinal fluid homeostasis through the regulation of specific ion and water channels in retinal macroglial cells and reduced subretinal fluid and choroidal vasodilation, with an improvement on the visual acuity in chorioretinopathy patients [1]. As another example, dexamethasone interacts with glucocorticoid receptors, activating anti-inflammatory pathways involved with cytokine production, inactivation of vascular cells and adhesion and transmigration of leucocytes into the site of injury [2], and it is useful, among other cases, in dry eye disease or uveitis [3]. In addition, 5-fluorouracil is a thymidylate synthase inhibitor with the ability to block thymidine synthase, an essential nucleoside for DNA replication and cell growth in proliferative vitreoretinopathies [4].

However, sometimes, as happens in the posterior segment of the eye, drug access is even more complex to reach the target site. Furthermore, in chronic diseases, the therapeutical drug concentration must be maintained over long periods of time. To solve these problems, controlled-release systems have emerged as the most promising alternatives. In the use of these new tools, drug administration is located near the target site, avoiding systemic undesired effects. Drug concentration can be maintained, and repetitive administration is not so frequent. The drugs are generally administered by invasive routes—the intravitreal is the most common—but as the number of injections is reduced, as are the undesired effects like high risk of infection, retinal detachment, endophthalmitis or even blindness, and poor patient compliance [5]. There are a wide variety of controlled-release systems in preliminary evaluation. For ocular disease treatment, solid ophthalmic devices, contact lenses, viscous liquids, gels, suspensions, colloidal systems (nanoparticles and nanosuspensions), matrix systems (ocular inserts, minitablets and collagen shields) liposomes, dendrimers, solid lipid nanoparticles, niosomes, and microparticles have been developed with varying success [6].

These systems incorporate the drug and then release it in a fixed way after administration. If the delivery system could reach its own specific target in the appropriate place where the drug must interact with its ligand, it would represent a step forward. The technology that allows the use of delivery systems as vectors is maybe the vastest and most laborious but promising path the pharmaceutical technology focused on controlled release could follow nowadays. This possibility is now a reality, for instance, with some kinds of cancer. To treat breast cancer, Abraxane® is a medicine for injectable suspension containing 100 mg of paclitaxel formulated as albumin-bound particles. These nanoparticles, with a mean particle size of approximately 130 nanometers, follow rapid dissolution after administration. Then, they bind to albumin-specific receptors on endothelial cells, which leads to activation of transcytosis (via vesicles), and release into the subendothelial space and tumor interstitium. Now, once the system is in the target place, the tumor uptakes the drug, and cancer cells die (for more information, visit www.abraxane.eu). This new approach shows at least two advantages over existing alternatives. First of all, it reduces the number of adverse effects, as the drug is only released on the target site and not on the entire systemic circulation. In addition, it is not necessary to achieve a certain systemic circulation, so the drug dose can be reduced and even personalized to each patient's requirement depending on the disease's degree and progression.

For ocular pathological treatment, there is also another difficulty. Access to this area from systemic circulation is not favored due to the presence of the retina pigment epithelium layer, the blood-retinal barrier and the efflux transporters, and from the anterior segment of the eye (after the corneal or non-corneal routes; it is not easy beforehand), the drug must gain access through the ciliary body, the trabecular meshwork and the blood aqueous barrier—too

many barriers that preserve the eye from foreign materials (Fig. 1). Despite these disadvantages, there have been some more or less successful attempts. In this review, the most interesting approaches for vectors specifically designed to reach target sites in ocular diseases are presented to understand the basis from which pharmaceutical technology could develop systems that can gain the authorities' approval and financing as first choices to cope with patients' needs.

2. DENDRIMERS' USE FOR SELECTIVE TARGETING

Dendrimers, globular, treelike branched nanostructured polymers (3–20 nm) [7], are a suitable candidate for a prodrug drug delivery and transfectant system design. Hydroxyl-polyamido-amine, or PAMAM, dendrimers have shown a histopathology-dependent retinal distribution. In ischemic retinas, OH-dendrimers are specifically localized in activated microglia and macrophages. The reason is not fully understood, but some authors refer to environmental changes, the increased phagocytic activity of activated microglia cells and macrophages, and the dendrimer's ability to readily partition itself to the retina from the vitreous chamber [8]. It cannot be ruled out that specific receptors expressed on activated macrophage-like cell lines, such as mannose receptors, could be target sites for dendrimers used as vectors [9] or cellular entry points, with internalization through endocytosis. Once in the target site, retention has demonstrated to improve by more than ten times (up to 21 days, in contrast with the 72 h needed for clearance in normal retinas).

Guo et al. [10] revealed an interesting behavior of novel 4th-generation cyanine-dye-conjugated PAMAM dendrimers. If these dendrimers are intravenously administered, they can easily reach microglia, Muller cells and retinal pigment epithelium cells that are in the area affected by optic nerve ischemia and inflammation in both murine and primate models of non-arteritic anterior ischemic optic neuropathy (NAION). Dendrimers accumulate in macrophages and activated astrocytes in the anterior optic nerve and in activated macrophages in the surrounding optic nerve (or extrinsic macrophages). It must be pointed out that these systems' uptake in healthy animals was minimal. Dendrimers remain for at least 30 days post-injection if they are administered soon after NAION. This could be related to disruption of the blood-brain barrier and the ability of dendrimers to access the anterior optic nerve and ischemic region. If dendrimers are intravitreally administered, they provide minimal access to the damaged optic region, showing different biodistribution, and only remain in the vitreous for 2 days.

3. "COATED" PARTICLES AS VECTORS

New particle elaboration methods allow location of proteins on the particle's surface. New polymers and block copolymers, such as the μ-RAFT agent and the tetraethylene glycol methacrylate (TEGM) selected by Whitmire et al. [11], can tether proteins to the surface when they are employed for microparticle elaboration. In this study, after polymerization of the amphiphilic block copolymer that renders particle formation by self-assembling technique, anti-inflammatory interleukine-1 receptor antagonist (IL-1RA) was located on the surface. The protein maintained structure and bioactivity. IL-1RA on the particle's surface can localize these systems on inflammatory mediator cells such as macrophages, neutrophils or B cells, and cause particles to exert two possible activities. First, IL-1RA blocks the IL-1 signaling pathway and modulates NF-kB activation after IL-1b stimulation and also enables particles to release the drug loading, i.e., an anti-inflammatory agent, in a controlled way. Both mechanisms of action could be useful in the treatment of surface ocular inflammation, as in dry eye disease and/or in ocular neurodegenerative diseases that are accompanied by inflammation.

The first data corroborated that the IL-1RA particles exhibited a significantly longer half-life in a sealed cavity, such as a joint, compared to the soluble protein (3.01 ± 0.09 days for IL-1RA particles vs. 0.96 ± 0.08 days for soluble IL-1RA). Similar behavior could be expected when administered in the posterior segment of the eye, a closed compartment surrounded by major barriers. However, if this system were loading with a drug, controlled release for a long period would not be expected. Only nanoparticles (300 nm mean particle diameter) were obtained. In spite of this, it may be possible to modify segment block copolymers' molecular weight to create larger particles with better-sustained release. Particles effectively bind to HIG-82 synoviocytes *in vivo* and inhibit IL-1b-induced signaling. Remarkably, the IL-1RA particles

inhibited NF-kB activation to the same levels as an equal amount of soluble IL-1RA.

To achieve this, the particles might have diffused through synovial fluid and tight junctions, which would be interesting behavior if administered in the ocular surface, reaching the anterior chamber, or in the posterior segment of the eye, crossing the vitreous and retinal barriers. Be that as it may, nanoparticles demonstrated being non-cytotoxic up to a concentration of 1 mg/mL in raw macrophages culture. No specific data for ocular toxicity were available.

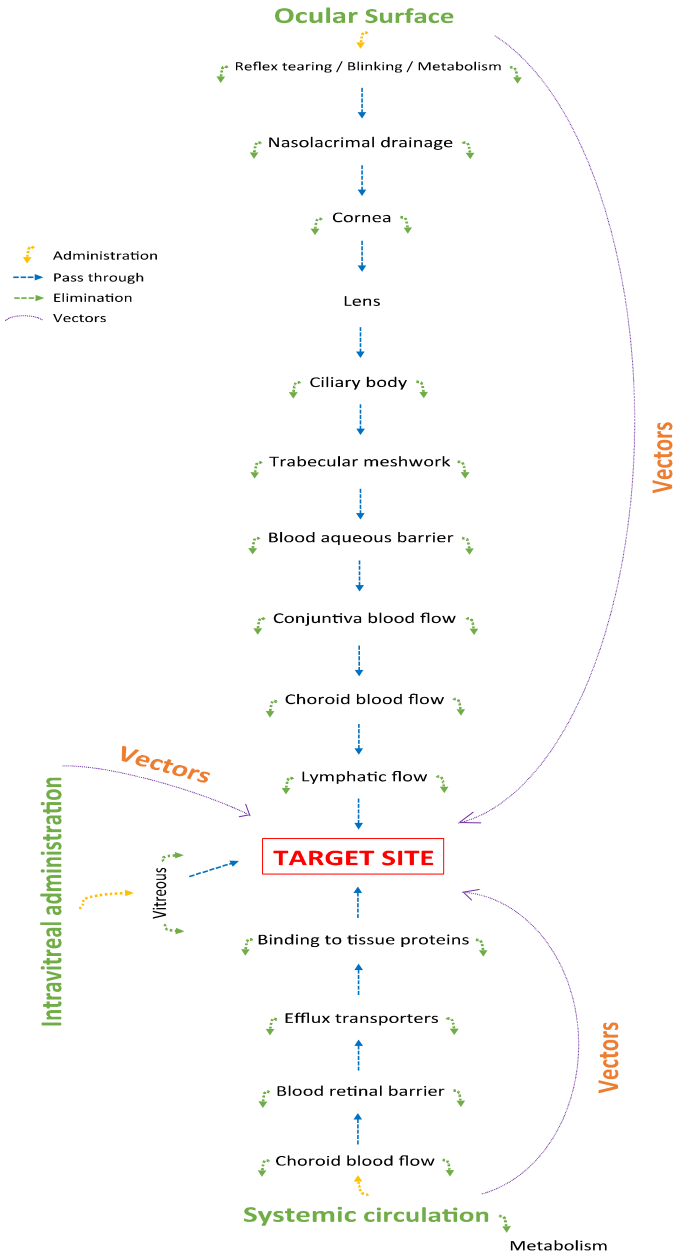

Fig. 1. Schematic representation of the main barriers a drug must overcome to access the posterior of the eye target sites. Barriers are shown from the ocular surface, from systemic circulation and after intravitreal injection

Table 1. First attempts for vectoring drugs into posterior segment of the eye target sites. The system design, its characterization and the proof of concept are included

System	Model	Administration	Target site reached	How?	Bibliography
PAMAM dendrimers	Murine (adult male Long-Evans rats, 200-250 g) and primate (adult male rhesus, 5 Kg) NAION model	- Intravitreal: 3 µL of 20 mg/mL D-Cy5 dendrimers dispersion - Intravenous: 20 mg dendr./kg rats 128 mg dendr./kg primates	Microglia, Muller cells and retinal pigment epithelium cells	Environmental changes; disruption of the blood-brain barrier	[10]
Nanoparticles	Adult male Lewis rats (200-250 g)	Intraarticular injection (27 G needle) 50 µL of µ-RAFT and TEGM microparticle dispersion containing 5 µg of IL-1RA	IL-1R on macrophages, neutrophils or B cells' surface	Diffusion through synovial fluid and tight junctions	[11]
Liposomes	Adult female Sprague-Dawley rats (225-250 g)	Single intravenous 0.5 ml injection containing ^{125}I-anti-CD163-liposomes ([mM] = 0.125)	Microglia, CD163+ macrophages	Capitation in peripheral level and infiltration	[12]
Non viral systems for gene vectoring	Adult female BALB/c mice (25-30 g)	Single intravenous 0.5 ml injection through the femoral vein containing 5 to 6 µg of plasmid DNA	Plasma membrane of retinal cells	Transferrin receptor expressed on blood retinal barrier cells allows capitation	[18]
	5-10 years old female Rhesus monkeys (5-10 kg)	Single intravenous 0.5 ml injection via the saphenous vein containing 70 µg of plasmid DNA	(I) Plasma membrane of retinal cells (II) Outer nuclear layer	(I) Transferrin receptor (II) Insulin receptor expressed on outer nuclear layer	[19]
	6-7 years old albino male Lewis rats (150-200 g)	Single intravitreal 5 µl injection containing 0.8 µg of oligodeoxynucleotide and 4.5 µg of VP22	Pigment epithelium retina cells	Specific electrostatic binding?	[20]
Viral systems for gene vectoring (AAV or EIAV)	Clinical assays - phase I/IIa	Intravitreal or sub-retinal injection	(I) Retina (II) Macula	Tissue tropism?	[22,26]

4. A QUESTION OF SIZE? PROMISING RESULTS FOR LIPOSOMES

Undoubtedly, access to biological structures is facilitated for reduced systems. Liposomes are controlled-release systems, which might be effective as vectors for retinal delivery of drugs. These systems can be developed with good loading efficacy of lipophilic drugs (higher than 90%) using different techniques, such as the ethanol injection method [12] or the film dispersion technique [13]. In ocular disease treatment, this is particularly interesting for dexamethasone, a corticoid available for the treatment of various ocular diseases and thus one of the most prescribed drugs worldwide [14].

Intermediate and severe age-related macular edema (AMD) pathogenesis and progression has been associated with changes in the number and location of retinal CD163+ infiltrating cells [15]. Treatments based on dexamethasone that specifically target these immune cells are promising and are a potentially revolutionary therapeutic tool in AMD. Despite no ocular results having been assessed, we must highlight the vectoring results obtained by Tentellier et al. (2016) because of the potential role this strategy could have in the development of new ocular pharmaceutical technology. A liposome formulation from a mixture of hematopoietic stem cells and progenitor cells, hydrogenated soy L-phosphatidylcholine, cholesterol and mPolyEthylene Glycol (PEG) 2000-PE in a molar ratio of 55:40:5 and loading with dexamethasone was prepared using the ethanol injection method to target CD163+ macrophages. Liposomes were sized by extrusion to achieve uniform distribution, and then a 0.125 mM liposome solution for intravenous administration was prepared. In this case, treatment with 0.02 mg dexamethasone/kg was administered in a 6-OHDA Parkinson's disease model in adult female Sprague-Dawley rats. The first dose of the liposome formulation was given 1 day before the intracerebral 6-OHDA lesion, followed by administration 3 times per week for 3 weeks. PEG incorporation plays two functions, first of all, acting as a coating barrier in which an anti-CD163+ antibody could be linked and also acting to ensure that the liposomes were not bounded by opsonins and unspecific uptaked in phagocytic cells.

As a result, dexamethasone delivery into the CD163+ macrophages was achieved, although the mechanism of infiltration is still not clear. Peripherally injected liposomes demonstrated the ability to target the CD163 receptor and to modify the local central nervous system microglia phenotype significantly (soluble mediators pattern) and achieve neuroprotection of dopaminergic neurons. Because the CD163+ population was specifically targeted, the dose needed was substantially lower than the normal dexamethasone doses used for anti-inflammatory purposes. If similar results were achieved in ocular CD163+ macrophages, as dexamethasone has been estimated to possess effective anti-inflammatory activity from a low concentration of 1000 ng/mL [16], the amount of the steroidal drug could be reduced significantly with the benefit of the lack of or the minimal possibility of adverse effects.

5. NON-VIRAL AND VIRAL GENE VECTORS, FIRST RESULTS FROM A CLINICAL TRIAL

Successful gene therapy requires a vector system that allows specific, efficient and long-lasting transgene expression in the target cells. Various non-viral (such as liposomes, lipoplexes, polyplexes and vectosomes [17]) and viral vector systems have been evaluated for cell gene transfer in the posterior segment of the eye. Among them, oligonucleotide vectosomes and the adeno-associated virus (AAV) have been proved to be the most suitable vectors for selective, safe, stable, efficient and long-term expression in the retina (including inner retina) and choroid.

Some creative oligonucleotide vectosome designs have been proposed to selectively achieve the retinal-pigmented epithelium. For example, Zhu et al. [18] prepared pegylated inmunoliposomes containing rat 8D3 monoclonal surface antibodies and double-stranded plasmid DNA – encoding bacterial β-galactosidase – driven by a cell-specific promoter in the interior to achieve two milestones. First, thanks to a monoclonal antibody that targets transferrin receptor-rich structures, such as the blood-retina barrier, the vector penetrates the posterior segment of the eye after intravenous administration. Second, but not least, the introduction of a selective promoter can control the expression of the plasmid DNA to occur only on cells of interest. A similar strategy was used by Zhang et al. [19], who compared their results with a new generation of pegylated inmunoliposomes coated with 83-14 murine

monoclonal antibody, an antibody with high affinity to the human insulin receptor. The results showed that the distribution of inmunoliposomes differed depending on the surface antibody. If 8D3 was used, there was an absence of gene expression in the outer nuclear layer, which was attributed to the minimal expression of transferrin receptors. Conversely, if 83-14 antibodies were employed, high transfection was observed since the outer nuclear layer expresses high levels of insulin receptors. The results of these studies confirm that it is possible to target different structures of the eye according to necessity. Finally, Normand et al. [20] demonstrated that a step forward consists of activating the system when it finally reaches the target site. VP22, a structural protein of the herpes simplex virus, was complexed with labeled antisense oligodeoxynucleotides, leading to the formation of nanoparticles. When injected in the vitreous body of rat eyes, it showed the ability to migrate through the retina to the pigment epithelium and accumulate. After internalization, the nanoparticles remained stable, and the genetic material only dissociated on illumination with white light, possibly due to thermal effects. Furthermore, the first assays revealed that a modulation of activation intensity might open the doors to personal medicine.

Among viral gene vectors, AAV stands out among other viral vectors due to its efficiency in transducing photoreceptors, Müller glia, retinal pigment epithelial cells and retinal ganglion cells [21]. Currently, the production of recombinant AAV vectors involves co-transfection of two engineered plasmids: (1) the transgene cassette that has removed all viral genes except for two palindromic inverted terminal repeats flanking the transgene of interest and (2) a helper plasmid that expresses viral replication and packages capsid genes necessary for replication [22]. Vector-loaded suspensions can be administered via a sub-retinal injection by pars plana vitrectomy and retinotomy with a 41-gauge cannula [23] or as an intravitreal injection, which is technically easier to perform and less invasive [24]. It is necessary for gene therapy that there be no toxicity, immunogenicity or changes in retinal responses on electroretinography.

These vectors have been proposed in gene therapy for complex ocular disorders associated with dystrophies and visual deficiencies. Such diseases are devastating and ultimately, in the absence of treatment, cause blindness. Among them, Leber's congenital amaurosis, an inherited retinal dystrophy, was the first ophthalmological disease targeted by a multidisciplinary approach including gene therapy. Phase I clinical trials have been carried out in 3 patients with AAV packing *RPE-65* gene [25] following mounting evidence of safety, successful proof-of-concept of visual improvement across multiple *RPE-65* animal models and the promising phenotype of *RPE-65* Leber's congenital amaurosis patients. The study demonstrated a relatively high safety profile of AAV-*RPE65* gene replacement without toxicity or dissemination of the vector, adverse surgical events such as intraocular inflammation or serious immunologic responses. More recently, equine infectious anemia virus (EIAV), a lentivirus, packed with gen *ABCA4* or *MYO7A* has been translated into clinical trials (phase I/IIa) for Stargardt dystrophy and Usher type 1B syndrome, respectively, with recent initiation of escalating doses [26]. For age-related macular degeneration, a phase I clinical trial for an EIAV vector able to express anti-angiogenic genes endostatin and angiostatin is being carried out in 40 patients [27]. Optimizing viral transduction, improving cell tropism and minimizing cell loss are the main challenges in the field.

6. CONCLUSION

The examples shown (Table 1) here are only the tip of the iceberg of the principles that can presumably guide the pharmaceutical technology over the next decades. It is too soon to brainstorm the future of controlled-release systems, but it is not difficult to imagine it involves the development of vectors thanks to their multiple benefits, further effectivity, security and, certainly, though maybe not in the beginning but sooner rather than later, economy. Promising results must be confirmed in future trials to assess the usefulness of vectors in the treatment of optic diseases, but, as we always said, the promising results reported will cause the pharmaceutical industry to focus deep attention on the development of a variety of controlled-release systems that can achieve drug vectoring, among other uses, for different ophthalmic therapeutic uses, and, as it is logical, it implies an increment of clinical trials.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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