

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

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.

Keywords Vectors; Controlled-Release Systems; Dendrimers, Nanoparticles; Liposomes; Posterior Segment of the Eye

1. INTRODUCTION (ARIAL, BOLD, 11 FONT, LEFT ALIGNED, CAPS)

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

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

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

61 For ocular pathological treatment, there is also another difficulty. Access to this area from systemic
 62 circulation is not favored due to the presence of the retina pigment epithelium layer, the blood-retinal
 63 barrier and the efflux transporters, and from the anterior segment of the eye (after the corneal or non-
 64 corneal routes; it is not easy beforehand), the drug must gain access through the ciliary body, the
 65 trabecular meshwork and the blood aqueous barrier—too many barriers that preserve the eye from
 66 foreign materials. Despite these disadvantages, there have been some more or less successful
 67 attempts. In this review, the most interesting approaches for vectors specifically designed to reach
 68 target sites in ocular diseases are presented to understand the basis from which pharmaceutical
 69 technology could develop systems that can gain the authorities' approval and financing as first
 70 choices to cope with patients' needs.

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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. (2016) 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. (2012), 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.

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 μ L 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	(Guo et al. 2016)
Nanoparticles	Adult male Lewis rats (200-250 g)	Intraarticular injection (27 G needle) 50 μ L of μ -RAFT and TEGM microparticle dispersion containing 5 μ g of IL-1RA	IL-1R on macrophages, neutrophils or B cells' surface	Diffusion through synovial fluid and tight junctions	(Whitmire et al. 2012)
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	(Teintillier et al. 2016)

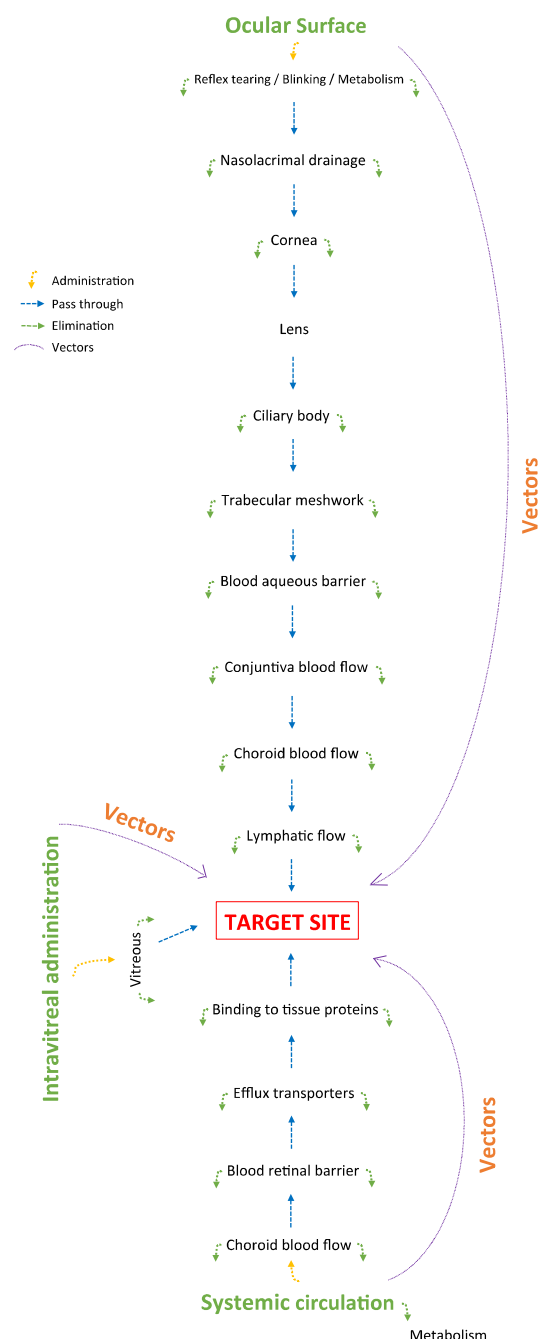


Fig. 1. Schematic representation of the main barriers a drug must overcome to access the posterior of the eye target sites. Barriers are shown from the ocular surface, from systemic circulation and after intravitreal injection.

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 [10] or the film dispersion technique [11]. 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 [12].

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 [13]. 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 uptaken 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 [14], 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. CONCLUSION

The examples shown 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.

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