Subretinal Injection: A Review on the Novel Route of Therapeutic Delivery for Vitreoretinal Diseases

Yingqian Peng Luosheng Tang Yedi Zhou

Department of Ophthalmology, The Second Xiangya Hospital, Central South University, Changsha, China

Keywords

Subretinal injection · Drug delivery · Intravitreal injection · Vitreoretinal disease

Abstract

Compared to intravitreal injection, subretinal injection has more direct effects on the targeting cells in the subretinal space, which provides a new therapeutic method for vitreoretinal diseases, especially when gene therapy and/or cell therapy is involved. To date, subretinal delivery has been widely applied by scientists and clinicians as a more precise and efficient route of ocular drug delivery for gene therapies and cell therapies including stem cells in many degenerative vitreoretinal diseases, such as retinitis pigmentosa, age-related macular degeneration, and Leber's congenital amaurosis. However, clinicians should be aware of adverse events and possible complications when performing subretinal delivery. In the present review, the subretinal injection used in vitreoretinal diseases for basic research and clinical trials is summarized and described. Different methods of subretinal delivery, as well as its benefits and challenges, are also briefly introduced.

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Introduction

Intraocular drug delivery is commonly used for the treatment of vitreoretinal diseases [1, 2]. In particular, anti-VEGF therapy consisting of intravitreal injection has been performed worldwide for the treatment of many vision loss diseases, such as age-related macular degeneration (AMD), diabetic macular edema, proliferative diabetic retinopathy, and retinopathy of prematurity, in targeting ocular neovascularization and vascular occlusions [3–7]. The treatment of intravitreal delivery is useful because it provides several benefits such as direct delivery of drugs into the vitreous and retina and the simplicity of achievement of this procedure by the medical doctors. However, this therapeutic method might also lead to adverse events and complications, such as intraocular inflammation, retinal detachment, ocular hemorrhage, and cataract, as well as patient noncompliance [2, 8]. Besides, because of the thickness and structure of the retina, intravitreal injection has a limited target effect in the posterior segment of the eye (e.g., retinal pigment epithelium [RPE] cells and photoreceptors).

Recently, subretinal delivery has becoming increasingly popular in both scientific research and clinical applications, and it has been regarded as one of the best strategies for gene therapy through subretinal local delivery of the

viral vector, which has been performed effectively for retinitis pigmentosa (RP) and Leber's congenital amaurosis (LCA) [9–12]. Also, it has been reported that subretinal injection of macrophages leads to pathological fibrosis, which could be used for assessment of advanced AMD in mice [13, 14]. Subretinal delivery can also be used for transplantation of stem cells in ocular degenerative diseases, which has been reported in *in vivo* studies and aimed towards clinical applications [15–18].

Compared to intravitreal injection, subretinal delivery has a direct effect on the resident cells and tissues in the subretinal space, while a higher stability and proficiency of operators are required. In this review, an overview of the benefits and challenges of subretinal delivery as a therapeutic option in vitreoretinal diseases is presented. Various advances and developments in subretinal delivery are also emphasized.

Overview of Subretinal Injection

Drug delivery in the eye is more challenging because of various ocular barriers compared to delivery of drugs to other parts of the body. Many of these barriers are considered to protect the eye from toxicants anatomically and physiologically, but at the same time they also makes drug delivery in the eye a challenging task for scientists and clinicians [19]. In general, ocular drug delivery includes 2 major parts: anterior segment drug delivery and posterior ocular delivery. For drug delivery in vitreoretinal diseases, like diabetic retinopathy and AMD, which are the most prevalent diseases affecting the posterior segment of the eye [20], an efficient way to deliver therapeutic material to the back of the eye is required. There are many unique barriers in the eye preventing optimal drug delivery into the retina, including ciliary nonpigmented epithelium (blood-aqueous barrier), RPE (outer blood-retinal barrier), and retinal vascular endothelium (inner blood-retinal barrier) [21]. Consequentially, the most convenient and patient-compliant route of drug administration – i.e., topical eye drops – is hardly effective for disease of the posterior segment of the eye, although it has been widely used in eye diseases, particularly for the treatment of anterior segment diseases [20]. Delivery of drug and gene or cell therapy to the back of the eye becomes an even more challenging task. In order to make drug delivery more efficient for vitreoretinal diseases, 2 major pathways have been developed to: intravitreal and subretinal drug delivery. Intravitreal drug delivery has been a dominant method in treating vitreoretinal diseases for the last few decades, and it

comprises direct intravitreal injection and intravitreal implantable device technology as well as bioerodible and nonbioerodible intravitreal implantable devices [22]. Via this effective technique, clinicians can administer drugs such as anti-VEGF [23–27] and steroids [28–31], among others, or gene therapy such as vectors containing specific genes [32–34], stem cells [35, 36], etc., directly into the back of the eye to increase drug concentration in the vitreous and the retina. However, the complications associated with this method have emerged, gaining increasing attention from clinicians. In spite of the common risks and complications caused by intravitreal injection itself, such as bleeding, retinal holes, and cataract, a high intraocular eye pressure is the most common one for steroids [37, 38], and it can lead to damage of the optic nerve and result in secondary glaucoma. Besides, endophthalmitis has been gradually recognized as a major complication of intravitreal anti-VEGF drug delivery [39, 40]. In addition, some scientists have pointed out that some retinal degenerative diseases mainly affect RPE cells and photoreceptors, such as AMD and RP. Thus, there should be another way to deliver drugs more directly to these cells. Under this circumstances, subretinal drug delivery has emerged and become increasingly popular for the treatment of vitreoretinal diseases.

The subretinal space, by definition, is the space between RPE cells and photoreceptors. In the subretinal space, injected material comes into direct contact with the plasma membrane of the photoreceptor, and RPE cells and subretinal blebs [9, 41]. This makes it an excellent site for drug delivery [42], especially in patients with vision-threatening disorders attributable to mutations in photoreceptor and/or RPE genes and retinal degenerative diseases.

In early times, subretinal injection was applied in a model of hereditary retinal degeneration (dystrophic RCG rats) to transplant human fetal RPE cells, and this provided the first indication that transplantation of human fetal RPE cells subretinally in rats was able to rescue photoreceptor cells [43]. Bindewald et al. [44] reported in patients with AMD the transplantation of RPE following CNV removal. They injected both heterologous and homologous RPE cell suspensions into the subretinal space and demonstrated preservation of foveal neurosensory functions [44]. For the past 10 years, subretinal drug delivery has been widely used in gene therapy and cell therapy experimentally and clinically for retinal degenerative diseases, such as AMD, RP, LCA, and Stargardt disease. The application of subretinal injections in both animal models and clinical trials is summarized in Figure 1.

Fig. 1. Applications of subretinal injections in both animal models and clinical trials. AAV, adeno-associated virus; LCA, Leber's congenital amaurosis; AMD, age-related macular degeneration; hESC-RPE, human embryonic stem cell-derived retinal epithelium; DR, diabetic retinopathy; BBS1, Bardet-Biedl syndrome type 1; EAU, experimental anotoimmune uveoretinitis.

Application of Subretinal Injection in Animal Models

Gene Therapy

The eye, in particular, is an attractive target for gene therapy for the following reasons: (1) insignificant immune responses will occur, (2) only small amounts of viral vectors might be needed to achieve therapeutic effects, (3) it allows localized treatment without intravenous delivery, and (4) the effects, efficacy, and safety can be easily observed and monitored by noninvasive technologies such as electroretinogram and optic coherence tomography [45, 46].

Adeno-associated virus (AAV) is a small, nonpathogenic dependovirus that has shown significant promise for safe and stable expression of a genetic payload in the retina [47], and it has emerged as the vector of choice for gene delivery to the retina. To date, AAV has been used as treatment in an increasing number of animal models of inherited retinal degeneration, and subretinal injection has been shown to be the most common and efficient way

to deliver AAV with target genes. Among all of the serotypes of AAV, AAV2 is well characterized and widely used.

In an LCA animal model, subretinal injection of recombinant AAV 2/5-OPTIRPE65 improved retinal function against retinal degeneration in Rpe65 knockout mice [48], and long-term transduction of RPE cells and photoreceptors in rats and nonhuman primates was observed [49]. AAV2 delivery of the RPE65 gene subretinally to the retina showed restoration of vision, rescuing the numbers of remaining photoreceptors both in young and old RPE65-mutant/deficient dogs [50, 51]. Watanabe et al. [52] reported on their subretinal delivery of 7 AAV serotypes into the developing mouse retina and demonstrated the possibility of gene replacement for the developmental disorder and subsequent degeneration of retinal photoreceptors induced by the absence of Crx. In retinal degeneration 11 (rd11) mice, which carry a mutation in the Lp-cat1 gene, subretinal injection of the AAV8 (Y733F)-smCBA-Lpcat1 vector was given on different postnatal

days and long-term preservation of electroretinogram responses as well as preservation of the retinal structure was observed, indicating that subretinal gene therapy in the LPCAT1 deficiency mouse model was successful [53].

In a neovascular AMD mouse model, subretinal AAV2 localization and plasmid protein expression was verified in the RPE and/or choroid of mice treated with all AAV2 constructs, and those authors suggested that subretinal injection of AAV2.COMP-Ang1 (AAV-mediated gene therapy with cartilage oligomeric matrix protein angiopoietin-1) has the potential to be an alternative and supplementary option to anti-VEGF agents for the long-term amelioration of neovascular AMD [54].

In a diabetic retinopathy mouse model, subretinal delivery of AAV2-mediated human erythropoietin (AAV2-CMV-hEPO) gene therapy was reported to be safe, and it could exert long-term protective effects on diabetic retinas [55].

In a Bardet-Biedl syndrome type 1 (BBS1) mouse model, in which severe retinal degeneration is one of the characteristics, BBSome formation and rhodopsin localization were rescued by subretinal delivery of AAV-Bbs1 (AAV vectors containing the *Bbs1* gene), and a trend toward improved electroretinogram results was shown [56].

In addition to AAV, other vectors, such as helper-dependent adenoviral vectors, which are considered to have a huge potential to improve the success rate of gene therapy achieved by the adeno-associated viral vector due to their large cloning capacity, have also been reported to complete gene delivery subretinally [57]. Lentiviral vectors are promising tools for treating retinal degenerative diseases such as AMD. Ikeda et al. [10] showed that retinal expression was stable in nonhuman primates via subretinal injection using simian immunodeficiency virus from African green monkeys (SIVagm)-based lentiviral vectors. Murakami et al. [58] reported that a newly developed lentiviral vector pseudotyped with Sendai viral envelope proteins (SeV-F/HN-SIV) achieved fast, efficient, and stable gene transfer in the mouse RPE when injected subretinally.

Based on the animal models described above, subretinal delivery of gene therapy has mostly focused on retinal degenerative diseases and gene deficiency diseases; with the target gene delivered subretinally by vectors, retina function can be restored to some extent.

However, not limited to retinal degenerative and gene deficiency diseases, AAV2-mediated subretinal drug delivery has also been used in experimental autoimmune uveoretinitis models in B10RIII mice [59–61].

As gene therapy via subretinal injection is a localized delivery to the subretinal space and the vectors are relatively safe, subretinal injection has been considered to be nonsystemic toxic, well tolerated, and effective in animal models [50, 62–64].

Cell Therapy

Another important application of subretinal delivery is cell therapy. The unique anatomy of the subretinal space makes it an efficient means of delivering cells to either target RPE cells or photoreceptors or both. In the past several years, various types of cells have been given subretinally in animal models for the treatment of retinal degeneration diseases. Pre-induced adult human peripheral blood mononuclear cells survived and migrated in retinal degeneration slow (rds) mice 3 months after subretinal transplantation [65]. In rats, photoreceptor degeneration can be prevented by subretinal transplantation of human fetal lung fibroblasts expressing the ciliary neurotrophic factor gene [66], and laser-induced choroidal neovascularization can be inhibited by subretinal transplantation of RPE overexpressing fibulin-5 [67]. Of all of the cell types, progenitor cells and stem cells, such as human neural progenitor cells [68], human retinal progenitor cells [69], progenitor cells from the porcine neural retina [70], forebrain progenitor cells [71], brain-derived precursor cells [72], human embryonic stem cell-derived retinal progenitors [73], human RPE stem cells [74], human bone marrow mesenchymal stem cells [75], and rat mesenchymal stem cells [76], are the most popular when given subretinally for cell replacement therapy for retinal degeneration. All these cells are considered to have the capability to survive and migrate into retinal layers and restore retina function or induce cell regeneration in different types of retinal cells when delivered via the subretinal route.

Cell therapy via subretinal delivery in animal models, especially using human embryonic stem cell-derived retinal pigment epithelium (hESC-RPE) cells, has been considered safe and nontoxic. It has been reported to be feasible and safe after subretinal implantation, with neither cell migration from the scaffold nor development of ocular or systemic tumors [77]. It therefore constitutes a promising start for human studies [78].

Application of Subretinal Injection in Clinical Trials

Since experiments and studies in animal models have demonstrated the feasibility and safety of gene and cell therapy via subretinal injections, a basis for clinical trials

has been built. There are several ongoing clinical trials using subretinal drug delivery-conducted gene or cell therapy to treat retinal degenerative diseases.

Gene Therapy

As early as in 2008, two clinical trials showed that delivery of a recombinant AAV carrying RPE65 complementary DNA subretinally is effective and safe as a gene therapy for LCA [79, 80]. In 2009, another clinical trial with 12 patients with RPE65-associated LCA confirmed the safety, extent, and stability of vision improvement in all patients who underwent subretinal injection of AAV2 containing a gene encoding a protein needed for the isomerohydrolase activity of the RPE, and it supported the application of AAV-mediated gene therapy for the treatment of inherited retinal diseases [81]. In recent years, long-term clinic trials have shown that LCA2 gene therapy provides stable visual and retinal function improvement after subretinal treatment [12]; the short-term results of a phase I trial demonstrated that both vector-related serious adverse events and systemic toxicities were not detected with subretinal injection of the AAV vector. Moreover, increased visual sensitivity was self-reported in all patients for treated eyes compared to control eyes, and this was remarkably noticeable under reduced ambient light conditions [82].

A clinical trial in *MERTK*-related RP patients showed that subretinal injection of rAAV2-VMD2-hMERTK, when administered carefully, may have resulted in clinical improvement in a subset of patients and it was not associated with major side effects [11]. In wet AMD patients, gene therapy with recombinant adeno-associated vectors delivered subretinally has been reported to be safe and well tolerated, and it can be used as a potential long-term treatment option for wet AMD [83, 84].

Cell Therapy

Cell therapy via the subretinal space has been developed for clinical applications. Schwartz et al. [85] reported the possibility of hESC-RPE to treat patients with Stargardt's macular dystrophy and dry AMD and provided preliminary data. Recently, 2 phase I/II studies with a length of 4 years, involving 18 patients with dry AMD or Stargardt's disease, demonstrated the possibility of safe implantation of hESC-RPE subretinally in an attempt to rescue photoreceptors and vision [86].

Although cell therapy via the subretinal space has been considered to be relatively safe when the right techniques are used and it is performed carefully, complications should still be a big concern when applying this technique

in a large number of patients. Recurrent retinal detachment with proliferative vitreoretinopathy was reported in a 60-year-old man with Stargardt's macular dystrophy after subretinal injection of autologous bone marrow-derived stem cells, but after retina reattachment visual acuity may be improved and return to the baseline [87].

Subretinal Delivery versus Intravitreal Injection?

It has been discussed that intravitreal injection provides greater direct drug concentrations to the vitreous and retina, which are commonly used for delivery of anti-VEGF agents, such as bevacizumab and ranibizumab in wet AMD patients [88, 89]. It is also useful for the delivery of triamcinolone acetonide, as well as antiviral drugs for ocular infections [90, 91]. However, regarding this delivery route, short-term complications including retinal detachment, endophthalmitis, and hemorrhage have to be noted [88, 91]. Moreover, intravitreal injection has a weak effect on some other drugs targeting the cells in subretinal spaces (RPE cells, for instance) because of the barriers (e.g., the internal limiting membrane) between the retina and RPE cells [92, 93]. It might also represent the injection of an unnecessarily high dose of the drug into the vitreous. Compared to intravitreal injection, subretinal delivery of viral vectors is thought to be the most efficient transduction of neurosensory retina and RPE [92]. It provides a direct route with more precise localization via a minimally invasive injection. With subretinal delivery, a lower dose is needed for accurate targeting of cells in the subretinal space.

The majority of studies and applications have focused on gene therapy using vectors, or stem cell therapy [94]. Gene therapy via vectors has been performed in studies for applications in inherited retinal diseases, such as RP and LCA [95–97]. Virus vectors might lead to acute toxicity and a cellular immune response, and the period of transfection gene expression is limited; it also has risks, i.e., potential safety hazards [98]. Unlike the vitreous cavity, the subretinal space is a closed anatomic area, which also has a greater immune privilege, and it could be a better and safer position for the delivery of virus vectors directly to RPE cells [99, 100]. Moreover, nonviral gene therapy has also been developed for ocular therapeutic purposes, which provides a wider range of applications, easier utilization, and lower costs, and it might increase efficacy and genomic safety [101].

Similar to gene therapy, stem cells have also been used for retinal degeneration diseases, and subretinal delivery

Fig. 2. Approaches of subretinal injections in animal experiments. **a** Transcorneal route through the pupil, passing the lens, vitreous, and retina. **b** Transscleral route entering the pars plana or limbus areas, crossing through the vitreous and the opposite side of retina into the subretinal space. **c** Transscleral route through the choroid and Bruch's membrane without penetrating the retina. RPE, retinal pigment epithelium.

provides a direct, effective, and safe route to specific targeting cells [85, 102]. Hu et al. [103] reported a new technique of subretinal implantation of ultrathin substrates containing stem cell-derived RPE cells, which showed a useful approach for stem cells and retinal transplantation studies.

Hillenkamp et al. [104] reported that in submacular hemorrhage patients with pars plana vitrectomy, subretinal delivery of tissue plasminogen activator (rtPA) with intravitreal injection of gas, rather than intravitreal injection of rtPA and gas, was more effective in displacement

of submacular hemorrhage. Subretinal injection leads to more frequent complications, such as retinal detachment, vitreous hemorrhage, recurrence of submacular hemorrhage, and postoperative development of CNV. Thus, a careful patient selection is necessary in order to ensure fewer complications after subretinal delivery.

Approaches for Subretinal Injection

Basically, approaches for subretinal injections can be divided into 3 groups: (1) a transcorneal route through the pupil, passing the lens, vitreous, and retina [105, 106]; (2) a transscleral route entering the pars plana or limbus areas, crossing through the vitreous and the opposite side of retina into the subretinal space [9, 107, 108]; and (3) a transscleral route through the choroid and Bruch's membrane without penetrating the retina [109–111] (Fig. 2). Regardless of the route chosen, all of the routes are effective for delivery of virus, viral particles, liposomes, plasmids, drugs, and formulations; they are also useful in collecting the contents of the subretinal space [41].

Scientists have demonstrated different approaches to complete subretinal injections as the anatomy of the eye-ball varies slightly according to the species and age. Usually this procedure is performed under direct visualization using an operating microscope, and a bleb formation should be observed as a sign of success of the procedure.

Since subretinal delivery is being increasingly used both in animal models and in clinical patients, new variations of this technique are emerged constantly. Mouse is the most common animal model for a large number of the inherited eye diseases which are perfectly suitable for gene therapy, and subretinal injections in mice are better studied and protocols of subretinal injections vary from group to group [94, 112]. There are also new approaches for other relatively larger animal models. A new platform device was reported to be helpful in relation to the monolayer of hESC-RPE implantation in retinas of rats [103]. A new subretinal injection device (RetinaJect Subretinal Cannula; SurModics, Inc., Eden Prairie, MN, USA) was evaluated and found to be superior to the conventional subretinal injection device when performing subretinal injection in dogs [113].

In the clinical procedures described above, the subretinal injections of the hESC-RPE reported by Schwartz et al. [85, 86] were preformed along the site of a pars plana vitrectomy, and the injection sites were carefully chosen on the basis of optical coherence tomography results. Sites with native, albeit compromised, RPE and similarly

compromised overlaying photoreceptors were thought to be the optimal injection spots for transplantation.

In sum, in order to achieve successful delivery of target genes or cells for the treatment of retinal degeneration disease via subretinal injections, suitable approaches should be considered depending on the species, age, available devices and the surgical skills of the physician.

Conclusion

In summary, subretinal injection is a novel route of drug and/or cell delivery for vitreoretinal diseases. In particular, subretinal injection provides better and safer ef-

fects in gene and cell therapies, and might be considered as a potential delivery option for personalized medical care with specific targets in the subretinal space.

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