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REVIEW ARTICLE

Gene therapy for hereditary ophthalmological diseases: Advances and future perspectives

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Abstract

Gene therapy is a promising new therapeutic strategy that could provide a novel and more effective way of targeting hereditary ophthalmological diseases. The eye is easily accessible, highly compartmentalized, and an immune-privileged organ that gives advantages as an ideal gene therapy target. Recently, important advances in the availability of various intraocular vector delivery routes and viral vectors that are able to efficiently transduce specific ocular cell types have been described. Gene therapy has advanced in some retinal inherited dystrophies; in this way, preliminary success is now being reported for the treatment of Leber congenital amaurosis (LCA). This review will provide an update in the field of gene therapy for the treatment of ocular inherited diseases. (Gac Med Mex. 2015;151:469-78)

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Introduction

Over the past two decades, molecular techniques have been developed to offer the possibility of a different form of transplantation therapy (gene therapy), in which a normal gene is "transplanted" within the cells of an affected person, thus enabling survival and normal function for the individual. Gene therapy is the release of DNA exogenous sequences known as transgenes, which are introduced into host cells, thus eliciting the production of proteins in response, making use of the transcriptional and translational structures of the host[1]. Cell transduction is a process by means of which cells are infected by introducing genetic material using different vectors such as viruses. Currently, some of the following methods are used as gene therapy to treat or to prevent specific genetic diseases:

- Replacing the mutated gene with a wild copy of it.
- Silencing a gene with abnormal function to avoid its expression.
- Adding or eliminating genes that are indirectly associated with the pathophysiology of the disease.
- Genetically correcting the sequence of the mutated gene by means of homologous recombination[2,3].

The eye offers many benefits as a target for gene therapy since it is an isolated, immunologically privileged and easily accessible organ[3-6]. The delivery of this treatment through different intraocular release routes (which allows for different types of cells to be manipulated), the reduced size and isolated structure

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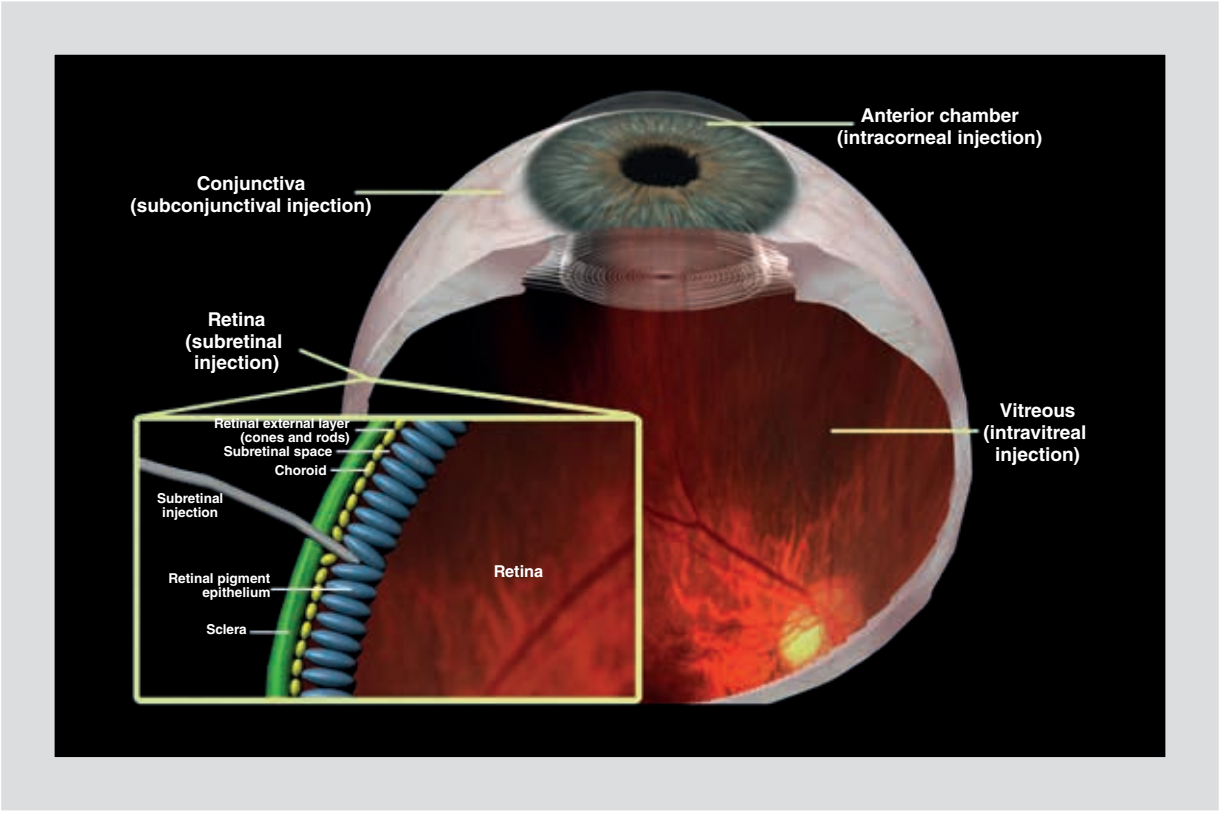

Figure 1. *Intraocular and periocular injection routes for viral vectors.*

of the eye, the use of different vectors that reach therapeutic effect with minimal systemic effects and the availability of studies with animal models[5], make this organ unique and essential for gene therapy studies.

This review will outline the routes for genetic material release within the eye and modern gene therapy strategies, mainly establishing the advantages and disadvantages of different viral vectors used. Subsequently, advances and achievements of gene therapy in the treatment of different human ocular diseases such as Leber congenital amaurosis (LCA), Stargardt disease (STGD), choroideremia (CHM) and age-related macular degeneration (ARMD) will be described.

Routes for genetic material introduction into the eye

The release of genes within the eye can be carried out by injecting vectors using different routes. The criterion for the choice of each one of them is based on the specific cell or ocular tissue that is intended to be modified, in adition to the characteristics of the used vector[3,6] (Fig 1.).

Injecting the vector in the sub-retinal space allows for the external cell layer of the retina, the retinal pigment epithelium (RPE) and, more specifically, the cones, to be transducted. This method is useful for the treatment of retinal degenerative diseases (LCA, STGD, achromatopsia and retinitis pigmentosa [RP]) caused by mutation in genes expressed in photoreceptors (PR) or RPE[3,7-9].

Introduction of the vector in the vitreal space enables transduction of the retinal most internal layers, such as Müller cells. This route is useful for the treatment of retinal neovascularization (as in the case of ARMD) or to prevent retinal ganglion cells death in the case of glaucoma, thus offering neuroprotection[10,11].

Direct injection in the anterior chamber allows for anterior segment tissues (cornea, iris, ciliary body, Schlemm's canal) to be transducted. For example, lentiviruses (LVs) have been used for the release of COX-2 (prostaglandin regulator) in episcleral veins to increase uveoscleral flow[12] and to decrease inflammation developing after corneal transplantation[13]. Spiga, et al. described a decrease of matrix metalloproiteinase (MMP-1) in the trabecular meshwork associated with the use of corticosteroids and with an increase in intraocular

pressure in experimental animals. In this study, intra-chamber injection of a recombinant adenovirus (AV) with human MMP-1 cDNA was able to reduce esteroid-induced intraocular pressure[14].

Transduction of periocular structures can be achieved by injecting the vector below the conjunctival membrane. This technique is useful for the release of antiangiogenic proteins in neovascular diseases of the choroid[15].

Gene therapy strategies

Recently, different strategies based on the introduction of genetic material in target cells of the body to replace, add, silence or correct genes directly or indirectly related to the pathophysiology of some hereditary diseases have been described[2].

Genetic replacement

It consists in the release of a gene which function is lacking due loss-of-function mutations in the affected gene. This strategy can be used in autosomal recessive disorders and in autosomal dominant disorders with haploinsufficiency– or dominant-negative effect producing mutations (e.g., RP) or in those where the supression of the mutated gene has already occurred, allowing for its correction by replacement[16].

Gene silencing

It consists in the release of a gene or nucleic acid to inhibit the expression of a gene or gene product with abnormal function. This strategy is useful in autosomal dominant diseases resulting from mutations with gain of function. Currently, it has been used to silence activated oncogenes, to suppress undesirable responses in autoimmune diseases or to inhibit pro-angiogenic genes expression[17,18]. Among these strategies, ribozymes[19], antisense oligodeoxynucleotides[20,21] and interference RNA[16,22], among others, have been used.

Gene addition

The objective of this strategy is to release a gene, the product of which provides beneficial effects regardless of the primary genetic defect. This therapy is employed for the treatment conditions resulting from a non-functional gene, as in the case of autosomal recessive diseases (e.g., congenital glaucoma, the *RPE65* gene in LCA), in X-linked RP (the *RPGR* gene) or ocular neovascularizations such as ARMD[1-3].

Gene correction

It is the release of nucleic acids to "repair" a mutated gene at its locus and restore its function. Genetic correction is performed by releasing the correct sequence of the gene with the purpose of inducing homologous recombination and replacing the sequence of the pathogenic mutation with a normal sequence. Within this strategy, there is other treatment possibility, which involves inducing an altered splicing, causing for the exon containing the mutation to be skipped[23]. This strategy is used on autosomal dominant or recessive diseases. Useful for *in vivo* transcription in autosomal dominant RP caused by mutations in rhodopsin[24,25].

Viral vectors

Gene release can be carried out using viral and non-viral vectors. Non-viral vectors include liposomes, synthetic polymers, DNA direct injection, interference RNA and electroporation, among others. All of them have the disadvantage of presenting low efficiency and a short-lived effect[2,3]. For these reasons, the method of choice is viral vectors, which have been modified to be non-pathogenic or replicative[1,3], while preserving sites to transport transgenic products that are inserted inside them. These viruses are able to infect the cells by releasing genetic material in their interior, something that is referred to as "cell transduction"[1], a process intended to produce stable and long-acting therapeutic molecules to replace those that are defficient or missing (Fig. 2). The most commonly used viral vectors are adeno-associated viruses (AAV), AVs and LVs[3,4].

AAV-type viruses

AAV viruses are simple DNA chain viruses and ideal vectors due to their ability and efficiency to transduct different cell types for long periods and with low immunogenicity[3-4]. Their limitation is their packaging capacity of 4.7 kb; however, new serotypes have been developed by means of which packaging is expanded up to 8.4 kb, which allows for large-gene diseases such as STGD to be treated. AAV vectors are currently being used in phase I/II clinical trials for gene therapy in different diseases, such as cystic fibrosis[26], alpha-1 antitrypsin deficiency[27], muscular dystrophies[28], Batten disease[29], Parkinson's disease[30] and have shown efficiency in hemophilia B[31] and LCA[32] patients. AAV-derived vectors are currently the most promising vectors

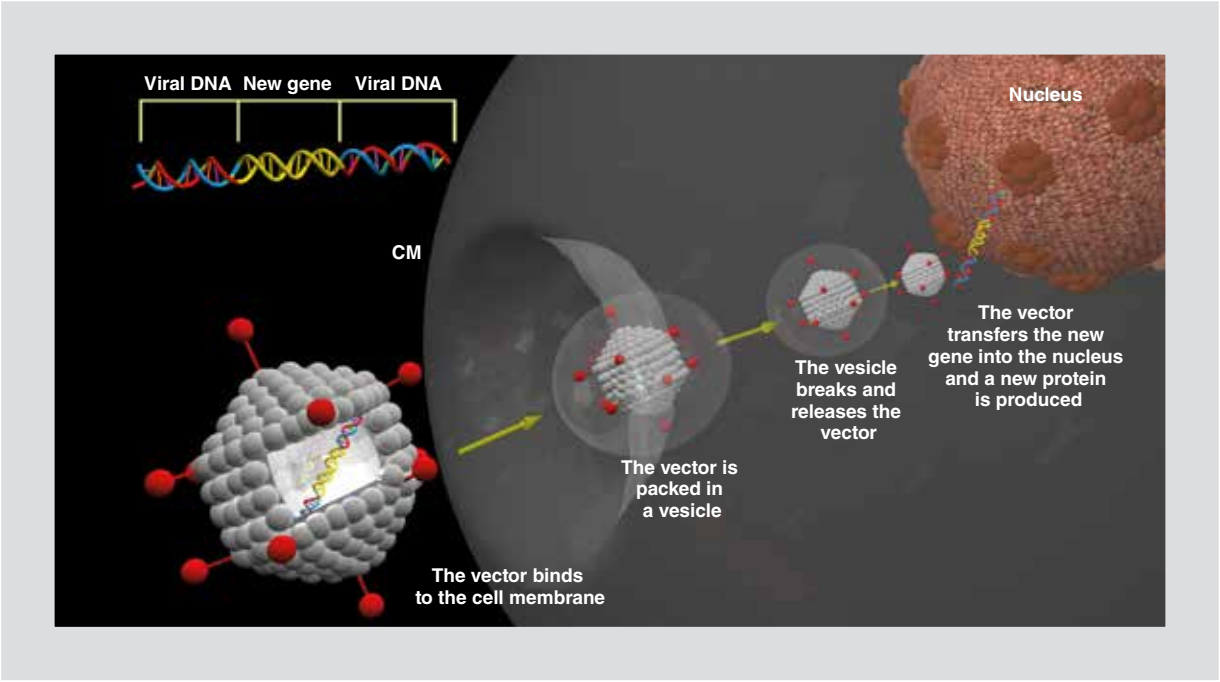

Figure 2. *Gene therapy using an adenovirus as vector. The "new" gene (that which has to replace the mutated gene) is inserted into a viral DNA (adenovirus, lentivirus). This way, the vector will be ready to be injected into a target cell with the purpose to produce a new, functional protein. CM: cell membrane.*

for gene release in the retina. Recently, sub-retinal administration of AAV2 has been shown to be safe and effective in patients with a rare form of hereditary blindness (LCA), suggesting that AAV-mediated retinal gene therapy can be successfully extended to other conditions severely affecting vision[33]. Moreover, this is supported by AAV great versatility as vector platform. Since there is a large number of AAV variants and many of them with unique transductional features, these vectors can be targeted to different cell types in the retina, including the glia, the epithelium and many types of neurons[34]. Currently, a series of these vectors have been designed *in vitro* to treat a variety of animal models with retinal pathologies[35,36]. This way, 11 serotypes and more than 100 variants have been described so far, out of which each one differs in the amino acid sequence, particularly those found in the hypervariable region of the protein capsid, which also results in gene-release properties differences for each variant[37]. For example, AAV2 vectors have sustained transduction in more than 50% of PRs, suggesting that this vector can be able to have a prolonged effect in the treatment of these degenerations.[38-40] Other vectors studied in murine models, such as AAV5, AAV8 and AAV9, have demonstrated that they can have a higher transduction efficiency rate and faster-onset transgenic expression[41-42].

LV-type viruses

These are lipid-enveloped viruses with double-stranded RNA, derived from the human immunodeficiency virus-1 (HIV-1) or the equine infectious anemia virus (EIAV). They are efficient in the transduction of non-dividing cells and long-term transgenic expression at the corneal endothelium, the trabecular meshwork and retinal tissues. Higher eficiency has been observed in the transduction of PR with EIAV than with HIV-1 vectors, as demonstrated in the study of STDG in animal models conducted by Kong, et al.[9]. These vectors have the capacity to pack up to 8 kb of genoma, which is immediately integrated to the chromosomes of the host cell.

AV-type viruses

These are double-stranded DNA viruses used for gene release in periocular tissues, anterior structures of the eye and in the retina. The great advantage of these short-lived transgenic products is that they could be used to destroy malignant cells as in the case of retinoblastoma, where AV vectors express herpes virus thymidine kinase, which turns ganciclovir prodrug into a triphosphate form that inhibits DNA replication and eliminates transducted cells[43]. Additionally, this vector does not integrate to the target-cells genome, which

reduces the risk for insertional oncogenesis. Its disadvantage include limited transport capacity and limited ability to transduct some retinal and vitreum cell-types, slow expression onset, in addition to not being useful for therapies requiring long-term gene transcription due to the immune reaction it triggers. Therefore, vector variants have been developed (e.g., helper-dependent Ad [HD-Ad]), which interrupt antigenic sites for immune evasion of the host and this way they allow for intraocular transgenic expression for up to one year after the vector is applied[44].

Ocular gene therapy in humans

Hereditary retinal degenerations

Hereditary retinal dystrophies make up a numerous group of phenotypically heterogeneous genetic diseases characterized by a progressive loss of PRs (cones and/or rods) and, consequently, of vision. These diseases affect ~1 of 2,000 individuals in the general population. To date, 221 genes and 261 loci associated with both isolated and syndromic retinal dystrophies have been identified[45]. Most of these conditions are caused by loss-of-function mutations and follow autosomal recessive, autosomal dominant or chromosome X-linked hereditary patterns[46].

Several characteristics make the retina an attractive target for gene therapy. Compared with most other organ-systems, the eye is small and compartmentalized, which enables the release of small amounts of gene vectors close to or at the very target site, for example, the sub-retinal space or the vitreal space. The anatomy of this compartmentalization and the presence of the blood-retinal barrier also limit the spread of the vector outside the eye, which reduces the severity of the immune response to the transference of these vectors. Another advantage of retinal therapy is that the targeted cell population is very stable and can afford sustained transgenic expression[46].

The first studies on gene therapy for retinal genetic diseases were conducted in animal models, and showed modest, but genuine improvements in some retinal cell-types function and survival. Over the years, the development of better vectors for retinal gene transference has led to better efficiency in the treatment of a wide range of animal models, leading to the initiation in 2008 of several clinical trials in LCA caused by *RPE65* gene deficiency. The results of these first two trials suggest that the treatment of hereditary retinal dystrophies based on gene therapy can be safe and effective[46].

First trials of gene therapy for retinal dystrophies

The first definitive study of gene therapy for a PR defect was carried out in 2001 in Rds mice, wich had null mutations in the *Prph2* gene (peripherin 2)[47,48]. The subretinal injection of AAV2 carrying the murine gene of Prph2 and a bovine rhodopsin promoter resulted in expression of the peripherin 2 protein at the external segment of the PR. This caused a restoration in the electroretinogram (ERG) to ~ 25% of levels in wild cells. However, in spite of the significant improvement in the PRs function in these mice, function duration was limited due to PR progressive degradation[48]. This partially successful rescue of these cells was followed by the first effective intervention of a retinal dystrophy caused by *RPE65* defects. This way, the most famous model is that of the Briard dog, which carries a natural deletion of 4 base pairs of the *RPE65* gene[49]. Sub-retinal injections of AAV2 in these animals potentiated an improvement of their ERGs, visual evoked potentials, pupillary response, as well as in vision-dependent movements and a functional return of the transductional pathway revealed by histological analysis of the PRs expression[50,51].

Gene therapy in humans with LCA due to mutation in RPE65

LCA is a congenital retinal dystrophy that causes severe loss of vision since very early ages. This condition affects approximately 1/81,000 individuals and up to 20% of children attended to in schools for the blind[52]. Careful observation and clinical description of LCA patients have revealed a spectrum of phenotypes and large variability in disease progression. All patients have early and severe visual deficit (before one year of age), attenuation or even absence of electroretinographic waves and absence of systemic data. The loss of visual acuity is deep and progressive since birth. Other associated ophthalmologic signs include the oculo-digital sign (eye “rubbing”), keratoconus/keratoglobus, cataract, strabismus, intra-retinal pigment migration, macular atrophy and optic nerve paleness[52]. To date, at least 22 retinal genes have been found to be associated with this dystrophy, most of which exhibit an autosomal recessive hereditary pattern, although an autosomal dominant pattern has been also described in rare occasions[53]. Night blindness is a symptom present in all LCA patients with *RPE65* mutations (LCA2). These subjects initially have poor vision and nystagmus. Electroretinographic records obtained

early during childhood have demonstrated residual function of the cones, and this is probably correlated with retinal architecture and cone preservation during the initial phases of the disease. Interestingly, visual function in LCA2 can improve during the first years of life to later deteriorate during the third to fifth decades of life[54,55].

The success of gene therapy in *RPE65*-deficient animal models for approximately 13 years, the restoration of visual function and a single dose applied in canine models have led to the conduction of 3 clinical trials, which are the first ones to use this type of treatment in hereditary eye conditions[56-58]. In a first study, Maguire, et al. described the efficacy and safety of gene therapy in three patients with LCA2 (from 19 to 26 years of age) in whom their right eyes (the most affected) were selected for recombinant DNA (AAV virus and RPE65 cDNA)-release surgery, whereas their left eyes were used as controls. Efficacy was monitored with objective and subjective vision measurements by comparing the average of at least 4 preoperative measurements with the average of at least 4 measurements taken 1 month post-injection. Objective measurements were pupillary reflection and the nystagmus test, whereas visual acuity exams, Goldmann visual fields and a mobility test to assess the patient's ability to walk in a poorly illuminated room with obstacles were included as subjective measurements[56]. The results demonstrated that all 3 eyes that received the injection had more effective conduction of the pupillary response (approximately 3 times higher than the baseline measurement at the beginning of the study), as well as a decreased frequency of nystagmus in primary and secondary positions and covering one eye[56]. Visual acuity, measured as the logMAR (logarithm of minimum angle of resolution), which can range from 0.00 to 2.00, showed logMAR improvements of 0.28, 0.45 and 0.34 for patients 1, 2 and 3, respectively. Additionally, in the Snellen chart, patients 1 and 2, who only recognized hand movement (0 letters on the chart), were observed to increase their visual acuity to 20/1,050 (approximately 2 lines) and 20/710 (22.5 letters or > 4.5 lines), whereas patient 3, who had a baseline visual acuity of 20/640, improved to 20/290 (> 3.5 lines)[56]. The test for ability to walk with obstacles was very complicated prior to the treatment, and the patients collided with most of the 14 obstacles and were diverted from their way in multiple occasions. After the injection, patient 2 was able to follow the lines of the pathway[56]. This study had no serious adverse effects. At the same time, Bainberg, et al. studied three young patients (17-23 years) with early onset and severe retinal dystrophy caused by mutations in RPE65[57]. The eye with the worst visual acuity in each individual was selected as the study eye, while the contralateral eye was used as control. This study demonstrated vision improvements as measured by microperimetry to dark adaptation, as well as by testing the ability to walk in a poorly illuminated room in a single patient at 6 months post-surgery. The study was also safe, and efficacy was demonstrated in one patient[57]. In a last study conducted in 3 patients, Hauswirth, et al. demonstrated (in an early follow-up 90 days post-injection) a significant increase ($p < 0.001$) only for visual sensitivity in poor environmental illumination conditions (sensitivity to dark adaptation)[58]. Simonelli, et al., in the cohort of 3 subjects reported by Maguire, described safety and efficacy 1.5 years after gene therapy was started[59]. In this report, the immune response continued to be good and there were no adverse events. The measurements of the pupillary diameter were quantified by the velocity (PLR velocity, PLRV) and amplitude (acceleration of pupil constriction, APC) of the pupillary light reflex and there was evidence that between days 150 and 415 there was a marked improvement in PLRV, which persisted up to day 545 (last cutoff day of the study). Significant p-values were identified when days 415, 365 and 305 velocities were compared in all 3 subjects. The ocular mobility test with videos demonstrated, in a follow-up of subjects 1 and 3 from day 60 on, that there was a decrease in monocular and binocular nystagmus in the primary position. Interestingly, patients 1 and 3, who had presented exotropia, also experienced a decrease thereof when interpupillary distance was measured[59]. Visual acuity continued to improve compared with previous reports. Thus, the logMAR score improved 0.21 (from 3 to 5 lines of letter in the eye at 50 cm from the chart), 0.19 (from 4.5 to 6.2 lines of letters in the eye at 50 cm of the chart) and 0.24 (from 8 to 10.4 lines of letters in the eye at 50 cm from the chart) in patients 1, 2 and 3, respectively. In the ability to walk in a dark room with obstacles test, all patients showed a slight, but continuous improvement in that year and a half year of assessment compared with day 30 evaluation[59]. Recently, Testa, et al., after a 3-year follow-up of 5 patients (out of which 3 were described by Maguire and Simonelly[56,59]), demonstrated a statistically significant improvement in corrected visual acuity between the baseline measurement and 3 years after therapy in the treated eye ($p < 0.001$) and the untreated eye ($p = 0.041$). In particular, maximum corrected visual acuity was observed 6 months after treatment in 3 patients and at 18 months in one. After that dates, all patients remained stable throughout all 3 years of treatment. One patient

who had a macular hole as an adverse event 14 days after initial treatment showed worsening of this sequel, but also an improvement and stability in visual acuity throughout all 3 years of therapy[60]. An increase in the field of vision area was also observed, with the largest being recorded at day 60 in 4 patients and at day 180 in one patient. This increase remained stable in all patients throughout all 3 years of treatment[60]. A statistically significant difference was observed in the percentage of constriction difference between the treated versus untreated eye in 3 patients in a cutoff at one year of treatment. Similarly, there was no significant difference in the percentage of short- and long-term pupillary contraction for any patient, which demonstrated that pupillary light reflection was stable in all 3 years of treatment[60]. A reduction in the frequency of nystagmus between the treated and untreated eye was also observed in all patients when baseline measurement and the period reported in this study were compared[60]. The ability of the subjects to walk across a dark room with obstacles (quantified by the number of obstacles avoided and the time taken to cross the room) remained stable during all 3 years. Average macular thickening, foveolar depression and retinal lamination remained stable in the optical coherence tomography (OCT) scan during all 3 years of follow-up[60]. A microperimetry test performed in one patient demonstrated a clear improvement in fixation stability in both eyes (treated and untreated) throughout all 3 years after therapy[60]. In conclusion, in clinical trials, gene therapy in LCA2 patients has demonstrated stability in the improvement of visual and retinal function, achieved few months after treatment.

Gene therapy for STGD: Stargen

STGD is the most common juvenile retinal degeneration, with a frequency of 1 in 10,000 individuals. This condition is characterized by a rapid deterioration of central vision, foveal RPE bilateral atrophy and frequent appearance of yellowish flecks in the macula and perimacular region of the retina. Different mutations in the ABCA4 gene are responsible of a wide range of retinal dystrophies, including RP, cone-rod dystrophy, ARMD and STGD[61].

In mice lacking copies of functional ABCA4, a delay in dark adaptation, an increase in all-transretinal after exposure to light, phosphatidylethanolamine (PE) in PRs external segment and excessive lipofuscin deposition in the RPE have been demonstrated[62]. Recently, Binley, et al. have demonstrated safety in the transduction of PRs with LV (EIAV) containing the ABCA4 gene in rabbits and macaque monkeys[63]. The release of a normaly functional gene in PRs bearing a mutation in the ABCA4 gene via gene therapy should be considered as a possible "cure" for ABCA4-associated diseases, since all these conditions are recessive, caused by a variable insufficiency that exceeds 50% of the gene's functionality (thus, adding a functional gene could completely restore functionlity) and, in addition, retinal cells degradation in all ABCA4-associated pathologies is late; therefore, this allows for a reasonable time window for a possible therapeutic intervention[9].

Based on animal studies, clinical trials using Stargen, a treatment based on LV carrying the ABCA4 gene, have been started. Currently, two clinical trials that accepted 28 patients are ongoing and are assessing the safety levels of 3 different doses (phase I/IIa). The only report so far refers that 8 patients have been treated at the first dose level with no serious adverse effects and that the study is going to proceed to the next dose level[64].

Gene therapy in patients with CHM

Choroideremia (CHM) is a X-linked retinal dystrophy with a prevalence of 1 in 50,000-100,000 individuals that is characterized by a progressive degeneration of the choriocapillaris, the RPE and the PRs. This disease is caused by mutations in the REP-1 gene that encodes for a protein involved in vesicular traffic[65]. In the first or second decade of life, affected individuals (males) start experiencing nyctalopia followed by restrictions in the peripheral field of vision and tunnel vision. In most cases, central vision is preserved until the age of 40-50 years. Paralleling the progressive changes in visual acuity, fine pigment changes appear, with focal choroidal atrophy and pigment spots, frequently described as "salt and pepper" in appearance. Degeneration progresses more centrally to include atrophy areas in the choroid periphery and the RPE. Atrophy in the macula is reported until the last stages of the disease[66]. Six male patients (35-63 years) were accepted in a clinical trial for phase I assessment and were treated with sub-retinal release of the AAV vector with the CHM gene[67]. In spite of having retinal detachment secondary to the surgical intervention, which usually reduces visual acuity, two patients with more advanced CHM who had a lower baseline corrected visual acuity gained 21 and 11 letters, respectively (more than 2 and 4 lines of vision), whereas the other 4 patients with normal baseline visual acuity had a minimal visual loss of 1 to 3 letters

at 6 months. Maximum sensitivity, as measured with microperimetry with darkness adaptation was increased in the treated eyes from 23-0 db at baseline to 25-3 db after treatment. Interestingly, in this study, the two patients with more severe CHM improved their visual acuity when baseline ophthalmologic exploration was compared with other 6 months post-therapy, whereas in the other 4 patients, visual function was preserved in spite of the surgical intervention, which will allow, with subsequent evaluations, for central acuity to be preserved and for macular degeneration, which is characteristic of the final stages of the disease, to be avoided or prevented.

Other clincal trials and preclinical studies with ocular gene therapy

Gene therapy in RP patients with mutations in the *MERTK* (human MER tyrosine-kinase receptor)

An example of gene therapy for retinitis pigmentosa (RP) in animal models that resemble the diseases in humans is therapy targeting mutations in the *MERTK* gene. The product of this gene is required for phagocytosis of PR external segments by the RPE, and when absent leads to a deep autosomal recessive degeneration of the retina[68]. Gene replacement studies have been conducted using AV, AAV and LV, where the use of the latter in the first studies was the more successful, since it was able to preserve retinal function for at least 7 months post-injection in rats[69]. Co-administration of lenti-Mertk and AAV expressing the glial cell-derived neurotrophic factor was more effective than lenti-Merkt alone[70]. More recently, vectors AAV8 and AAV2 showed constant retinal function and safety in rats after treatment[71,72]. Owing to this, a phase I clinical trial using an AAV2 vector with a RPE-specific promoter containing MERTK has been started in Saudi Arabia and, to date, 3 patients have been treated with a subretinal injection with no adverse effects recorded so far[68].

Gene therapy in patients with Usher syndrome (USH)

USH is a heterogeneous group of autosomal recessive diseases characterized by deafness and blindness. Three forms have described that are differentiated by deafness severity and progression with or without vestibular dysfunction and RP[73]. USH1 is the most common form, and mutations in at least 5 genes are associated with this disease, with the MYO7A gene being responsible for 60% of mutations in this subtype[73]. Studies in mouse models (shaker1 mouse model) have demonstrated that EIAV-MYO7A sub-retinal injection is able to express in RPE and PRs[74,75]; similarly, this sub-retinal injection has been shown to be safe and well tolerated in macaque monkeys[75]. For the above reasons, a phase I clinical trial is underway in the United Kingdom using the lentiviral vector (EIAV) to assess the safety of MYO7A sub-retinal release in patients with USH1B[68].

Gene therapy in patients with ARMD

The vascular endothelial growth factor (VEGF) is the most important pro-angiogenic factor promoting neovascularization of the choroid vasculature in age-related macular degeneration (ARMD), which is the main cause of blindness in people older than 65 years[76]. Recently, an AAV2 vector with a new soluble chimeric protein (AAV-sFLT01) controlled neovascularization after a single injection in a murine model when it was released by the intravitreal route, a less invasive route than sub-retinal administration[77-79]. Subsequently, Lukason, et al. included the analysis in non-human primates in a neovascularization model, with expression being observed for 5 months in the aqueous humor[8]. Based on these data and the safety demonstrated in monkeys, Genzyme/Sanofi has started a phase I clinical trial using intravitreal AAV2-sFLT01 for ARMD. Vitreal injection of AAV-endostatin, AAV-angiostatin or angiostatin lentiviral vector has also demonstrated to significantly decrease choroidal neovascularization. In part, these data have lead to the phase I of a clinical trial using a lentiviral EIAV vector that expresses endostatin (a product of collagen XVIII metabolism) and angiostatin (a product of fibrinogen metabolism), both inhibitors of angiogenesis.

Gene therapy in patients with hereditary ophthalmologic diseases

To date, there are many preclinical models being studied, including *GUCY2G* (LCA), *GNAT2, CNGB3* (achromatopsia), *MFRP* (autosomal recessive nanophthalmos-RP), among others. In Mexico, Zenteno, et al. described a new autosomal recessive syndrome characterized by nanophthalmos-RP-foveoschisis-optic nerve drusen in two Mexican[81,82] and one Spanish families[83]. This syndrome starts at the second or third decades of life and the authors searched for mutations

in the *MFRP* gene because it has been associated with nanophthalmos in previous reports and because clinical data consistent with recessive retinal degeneration were found in a *MFRP (rd6 MFRP)*-deficient animal murine model. Homozygous mutations were identified in all studied families, including one of the compound heterozygous-type. These findings are supported by Won, et al., who demonstrated that this gene is necessary for the preservation of PRs[84]. In a study conducted in a mouse model with early onset of pathological features, sub-retinal release of an AAV8 vector containing *MFRP* cDNA on post-natal day 14 was able to prevent PR degeneration and restore the function in the rd6 mouse (at 2 months post-injection, assessed by ERG and retinal histology), indicating that the model can be useful for gene therapy in some clinical trial[85]. Recently, using an AAV2 vector modifying that of the previous report[85], gene therapy was also shown to effectively delay PRs degeneration, but not as effectively as in the previous study[86].

Conclusions

A notorious progress has been achieved in the understanding of hereditary ocular diseases pathogenesis and in improving safety and specificity of ocular genes transference using certain vectors. Preliminary successes have been reported in phase I clinical trials conducted for conditions such as LCA, STGD and ARMD; nevertheless, further experimental studies are needed in animal model eyes for other conditions in order to translate them into clinical trials and thus bring hope to many patients, with the purpose to offer them potential ocular therapies in the near future.

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