Application of Canaloplasty in Glaucoma Gene Therapy: Where Are We?

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Abstract

Purpose: Schlemm's canal (SC) inner wall is adjacent to the juxtacanalicular trabecular meshwork (TM) over their entire circumference. We seek to transfer reporter and therapeutic genes to these outflow-modulating tissues via canaloplasty surgery in live monkeys.

Methods: A standard canaloplasty surgical approach was performed in cynomolgus monkeys using flexible canaloplasty catheters, modified for monkey eyes with a 175- μ m outer diameter and an LED-lighted tip. A 6-0 prolene suture was used for the exact localization of SC. Trypan blue was injected during catheter withdrawal to document catheter placement within SC and to determine ease of injecting fluid into SC. Before, during, and after the injection, the position of the catheter and the anatomic details were video-captured with an externally positioned noncontact endoscopic imaging system and 50 MHz ultrasound biomicroscopy (UBM).

Results: A 360° catheterization and injection of dye into SC was achieved. Suture, catheter, and trypan blue were imaged with the endoscope camera system and the catheter was also visualized with UBM. Trypan blue was seen in the SC over 5 clock hours after a 1 clock-hour insertion of the catheter.

Conclusions: A modified canaloplasty catheter device might be used for gene delivery to the SC/TM area without circumferential catheterization. Further studies comparing different delivery methods of the vector/transgene into the SC using canaloplasty are needed.

Introduction

PRIMARY OPEN-ANGLE GLAUCOMA (POAG) is a chronic, progressive optic neuropathy. Increased intraocular pressure (IOP) is a major and modifiable risk factor for POAG. Thus, decreasing IOP using medical and surgical treatment methods is the mainstay of glaucoma therapy. Medical treatment strategies are noninvasive but require the patient's adherence to self-administration of topical eye drops. Although surgical approaches are more effective in terms of decreasing IOP, they have their own vision-threatening surgical risks, such as hypotony, bleb leaks, choroidal effusions, maculopathy, or endophthalmitis.¹ Trabeculectomy is still the gold standard among surgical methods, but nonpenetrating surgical approaches, such as viscocanalostomy, deep sclerectomy, or canaloplasty, are alternative ways to avoid the potential vision-threatening complications of trabeculectomy. Minimally invasive glaucoma surgery, including trabecular microbypass stent, trabectome, Schlemm canal scaffold, and excimer laser trabeculostomy, has also gained popularity in recent years.²

Increased IOP in POAG results primarily from high flow resistance in the juxtacanalicular trabecular meshwork (TM) and the inner wall of the Schlemm's canal (SC).³ In recent years, the possibility of decreasing this outflow resistance via gene therapy using TM/SC-targeted transgene delivery systems has generated considerable interest.⁴⁻¹⁶

We hypothesized that a canaloplasty approach might also be used for localized transgene delivery into this region. In this article, we have briefly reviewed canaloplasty surgery and discuss its possible role in transgene delivery into the SC.

Viscocanalostomy and Canaloplasty

Canaloplasty is a nonpenetrating glaucoma surgical approach that aims to use a patient's natural outflow pathways with fewer surgical complications compared to trabeculectomy. Canaloplasty is a modified viscocanalostomy in which a catheter device is used to cannulate SC and to inject viscoelastic material circumferentially.^{17,18} In viscocanalostomy, superficial and deep scleral flaps are performed to unroof SC.

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Injection of the viscoelastic material through the ostia of SC on each side and dissection of the deep flap leading to formation of intrascleral lake constitute the main steps of this surgical procedure. Following these steps, aqueous humor may percolate through Descemet's membrane into the SC through the ostia and leave the eye via the normal collector canals. Fluid may also leave the eye via the small microruptures in the inner wall of SC that were created by the injected viscoelastic material, or via the uveoscleral outflow pathway.¹⁸ Viscocanalostomy has been shown to decrease IOP in patients with POAG and also increase outflow facility in nonhuman primates.^{17,19}

Canaloplasty uses the same methods to unroof SC, whereafter a flexible microcatheter with a lighted tip is inserted into one ostium and passed circumferentially to dilate and stretch SC until it exits the other ostium. A 9-0 or 10-0 monofilament suture is then tied to the catheter tip and passed through the SC as the catheter is being withdrawn. Finally, the suture ends are tied outside SC in order to increase SC inner wall/JCT deformation and thereby increase trabecular (conventional) outflow facility.^{20,21}

Various studies have shown that canaloplasty surgery reduces IOP more than does viscocanalostomy.²² As a nonpenetrating glaucoma surgery, canaloplasty, like viscocanalostomy, has fewer surgical risks compared to trabeculectomy.²⁰ However, it reduces IOP less effectively than does trabeculectomy,^{23,24} and thus is usually reserved for patients with mild-to-moderate glaucomatous damage who do not require single-digit IOP.

Gene Therapy for Decreasing Outflow Resistance

In recent years, ocular gene therapy has been a popular research topic, since it might provide a long-term treatment option for chronic ocular diseases.⁴⁻⁸ For glaucoma, various genes have been investigated (dominant negative Rho or Rho kinase, caldesmon, C3 transferase, matrixmetalloproteinases, and specific siRNAs) for efficacy in decreasing outflow resistance.⁹⁻¹² These genes are thought to modify the structure of the TM that is responsible for the outflow resistance. Prostaglandin pathway gene therapy has also been investigated and found to produce sustained reduction of IOP in domestic cat and nonhuman primate models.^{13,14} Gene delivery techniques include nonviral (naked DNA injection, physical, and chemical approaches) and viral (herpes simplex viruses, lentiviruses, adenovirus, and adeno-associated virus) systems.⁹ Viral methods have induced transgene expression in cultured TM cells and/or the TM of live animal eyes.⁹ Viral vectors (FIV and scAAV) encoding genes for green fluorescent protein (GFP) show long-term (2+ years) expression in the outflow pathways of nonhuman primate eyes following transcorneal intracameral injection.^{15,16}

Transcorneal intracameral injection has been the method of choice for transferring transgenes/vectors into the anterior chamber (AC) of the eye. Successful transgene expression has been achieved using this route in rats, cats, and monkeys.^{5,13-16,25,26}

Disadvantages of the intracameral route for transgene injection are the potential for clinically significant inflammation and possible transgene expression (a concern with high virus titers) in other anterior segment structures, such as the cornea and iris.¹⁶ With most drug delivery methods,

for example, topical drops, implants, and others, nontarget tissues are exposed to the active compound. There are a number of tissues in the anterior segment in addition to the TM/SC that would be good targets for glaucoma gene therapy. The ciliary body has potential for several applications—the ciliary muscle for PG-related gene transfer aimed at increasing uveoscleral outflow^{13,14,16} and the ciliary processes for carbonic anhydrase inhibitor or beta-adrenergic receptor-related gene transfer aimed at inhibiting formation of aqueous humor. The question of off-target effects will be an important issue for these and other gene therapy applications moving forward.

The use of canaloplasty for delivering transgene/vectors in the TM/SC region might reduce potential unwanted side-effects and also let us deliver vectors selectively to a location directly involved in outflow resistance. Other advantages to using canaloplasty include injecting smaller volumes and/or lower titers of vectors and delivering transgenes to the entire circumference of SC and the JCT region of the TM.

There are some drawbacks to using this technique. It is a much more complicated and difficult procedure to perform than intracameral injection and requires an experienced ophthalmic surgeon; there is a risk of postsurgical complications and it cannot be repeated frequently as can intracameral injection. Another challenge might be the physiological pressure gradient between AC and SC and normal aqueous flow to SC via the TM. A peripheral corneal paracentesis performed at the beginning of the surgery might provide a reverse flow and let the viral particles diffuse from SC to the TM. Since aqueous humor formation is a dynamic process, the physiologic pressure gradient should return to normal levels in a short time, increasing the exposure time of the TM to the viral vector.²⁷ A recent study graded suffusion of fluorescein BSS solution from SC to AC during catheterization for canaloplasty after IOP was lowered below episcleral venous pressure by paracentesis in POAG patients.² They classified the suffusion into 3 grades: (1) poor suffusion with spread < 1/8 of the corneal diameter in the AC, (2) moderate suffusion with a spread between 1/8 and 1/4 of the corneal diameter, and (3) extensive suffusion with a spread between beyond 1/4 of the corneal diameter. The authors believe that grade 1 suggests a pathologic TM. Thus, it also suggests that viral vector delivered by canaloplasty might not reach the iris, cornea, and the lens in eyes with glaucoma and increased trabecular outflow resistance. Lowering IOP by paracentesis before the vector injection into SC, especially in glaucomatous eyes with a pathologic TM, may limit the entrance of virus into the AC.

In the current study, we tested whether TM/SC-targeted gene therapy might be possible by using canaloplasty.

Canaloplasty for TM/SC-Targeted Gene Therapy—Where Are We?

Experimental history and methods

Before starting the sequence of surgical experiments, microcatheters especially designed for monkey eyes, with outer diameters of 150 and 175 μm and inner diameters of 125 and 100 μm , respectively, were obtained (iScience Interventional, Menlo Park, CA). All animal experiments were performed in accordance with the Institutional Animal Care

and Use Committee approvals and the Association for Research in Vision and Ophthalmology (ARVO) Statement for the Use of Animals in Ophthalmic and Visual Research.

Initial experiments to work out surgical, catheter, and other technical details used enucleated eyes from 3 cynomolgus (*Macaca fascicularis*) and 3 rhesus (*Macaca mulatta*) monkeys. The first set of eyes obtained for these experiments were from a rhesus monkey and were fixed. It was not possible to thread the catheter in these eyes, likely because the tissue was not pliable, as normal unfixed tissue would be, and there were concerns about catheter diameter. The 150- μ m catheters were then obtained but to achieve the smaller outer diameter the light source had to be a separate piece on the inside of the catheter and had to be removed before injections could take place. The second set of experiments used 1 cynomolgus and 1 rhesus. In these experiments the 150- μ m catheter was attempted but the 175- μ m catheter was preferred because the light source did not have to be removed for injections. The 175- μ m catheter was threaded through several clock hours in both species. In the third set of experiments, using 1 cynomolgus and 1 rhesus, it was determined that using a 6.0 prolene suture to locate SC and determine patency 360° was a useful step. It was also determined that using the standard 27 ga cannula was not

optimal for Healon GV injection and that modifying the 175- μ m catheter to allow insertion of a 33 ga cannula was a superior method for injecting Healon GV into SC to expand it for catheter insertion. It was also determined during the third set of experiments that the 175- μ m catheter would fit in both rhesus and cynomolgus eyes and that the light source was not necessary for successful catheterization. The 150- μ m catheter may still be necessary for use with smaller eyes, and comparisons of the inner wall of SC postcannulation need to be made to assess structural changes that might be due to catheterization.

Once the surgical technique was refined, a fourth set of experiments was initiated using 2 live monkeys (1 cynomolgus and 1 rhesus). Standard steps of canaloplasty surgery were performed (Supplementary Video S1; Supplementary Data are available online at www.liebertpub.com/jop). Superficial and deep scleral flaps were made at 12 o'clock and the scleral spur and SC were located. Aqueous percolation was observed. Before entering SC, sodium hyaluronate (Healon GV; Abbot Medical Optics, Inc.) was injected into the AC via a 30 ga blunt cannula, through a paracentesis made with a No. 75 sharp-tipped razor blade, to deepen the AC and improve endoscopic visualization of the angle structures. Healon GV was injected into SC through a

FIG. 1. (A) Endoscopic camera image showing the 6-0 prolene suture in the Schlemm's canal (black arrow) and its end (dotted arrow). (B) Microcatheter in the Schlemm's canal (arrow). Note the blood in the canal blocking the gonioscopic view of the catheter (on the right side of the picture). (C) LED light of the microcatheter in the Schlemm's canal. (D) Endoscopic camera image of the temporal side of left eye at 3:00–4:00 showing the trypan blue in Schlemm's canal after the injection by catheter (ostium was at 12:00, the catheter tip at 1:00). (E) The infero-temporal site at 3:00–4:00 and the point where the visible dye column ended (arrow) at 5 o'clock.

33 ga Grieshaber cannula at the beginning of the cannulation. A 6-0 polypropylene suture (Prolene, Ethicon, Inc.) was passed all the way around SC and the specially designed microcatheters were then used to cannulate SC.

At different stages of surgery, in both enucleated eyes and live animals, the angle and SC were visualized using a Swan-Jacob gonioscens (Ocular Instruments), an endoscope system²⁸ using a Nikon D1 × digital camera combined with a 175-W xenon nova light source and a 3-mm × 6-cm 0° teleoscope probe (Hopkins II; Karl Storz Endoscopy-America, Inc.) and/or a 50 mHz ultrasound biomicroscopy (UBM) (Humphrey model 840; Carl Zeiss Meditec). UBM images were recorded to tape and/or smartphone.

During the live-monkey surgeries, anesthesia was induced with ketamine HCl (15 mg/kg IM); surgical plane anesthetic depth was achieved with inhalation of anesthetic isoflurane 2%. At the end of the surgery, animals were sacrificed with pentobarbital Na (25–30 mg/kg IV) and then perfused transcardially with 3 L of 0.1 M phosphate-buffered saline followed by 3 L of paraformaldehyde 4% for 10 to 15 min.

During both live-animal experiments, preoperative anterior segment and gonioscopic examinations of all eyes were normal. However, the animals were donated by the Primate Center; postsurgical evaluation of IOP was not allowed and they had to be sacrificed immediately. Thus, preanesthesia, postanesthesia, and pre-euthanasia IOPs were not measured.

In the first live-animal eye, the 6-0 prolene suture was passed 360° and was visualized using the endoscope system (Fig. 1A). In the fellow eye of the same animal, a small volume of Healon GV was injected into each side of the ostium using a 33 ga metal cannula and the 175-μm microcatheter was inserted into SC and then passed nasally through 6 clock hours. The catheter was then reinserted in the other ostium of SC and passed temporally to nasally all the way between 12 and 9 o'clock but did not move forward beyond that point. Using the endoscopic camera system the catheter was easily visualized (Fig. 1B). During the gonioscopic examination no iris hemorrhage, iris root tears, or other evidence of inner canal wall rupture were observed, and the pupil remained round. At that point, the injection catheter was then shortened and trypan blue was slowly injected into SC through the 33 ga metal cannula attached to the injection catheter and microcatheter system, while the microcatheter was being pulled back, starting from 9 o'clock. Injection of the trypan blue was easily achieved and was seen throughout the entire circumference of SC although the microcatheter did not move forward from the 9 o'clock position. Dye in SC adjacent to scleral spur and TM was well visualized with the endoscope system. The LED light of the microcatheter was difficult to see under the bright lights of the endoscopic camera and the operating microscope but it could be visualized from the side (Fig. 1C).

In a second live-animal experiment, the goal was to investigate how far trypan blue could go in SC after the microcatheter was pushed forward just 1 clock hour from the entry ostium since trauma to the walls of SC caused by the microcatheter might be a problem in terms of gene expression. The microcatheter was inserted a single clock hour from 12 to 1 o'clock and dye was injected at 1 o'clock. The dye ended in SC at approximately 5 o'clock, as estimated from the endoscope images, indicating that dye could pass at least 4 clock hours beyond the microcatheter tip (Fig. 1D, E).

FIG. 2. Ultrasound biomicroscopic picture showing the microcatheter in the Schlemm's canal as a hyperechoic spot (arrow) (A) and the fellow eye with normal appearance in the corresponding area (B).

The presence of the cannula inside SC was also clearly detected by UBM (Fig. 2A, B).

Discussion

Glaucoma is a chronic disease. Current treatments to slow or stop disease progression include long-term topical ocular medications and/or surgical/laser treatments. Issues with these therapeutic strategies include ocular surface toxicity, patient noncompliance with topical drop regimens, and surgical complications. Thus, long-term treatments using gene therapy technologies to provide sustained delivery of a therapeutic gene into the eye, effectively removing the patient from the drug delivery system, would definitely be useful, especially in developing countries.

To the best of our knowledge, this is the first study to report cannulation of SC in cynomolgus monkeys. Tamm et al.¹⁹ performed viscocanalostomy surgery for the first time in rhesus monkeys. We performed our initial experiments in enucleated rhesus and cynomolgus eyes and our live-animal experiments used both a cynomolgus and rhesus monkey. Although cynomolgus eyes are smaller than rhesus eyes, they can successfully be used for canaloplasty experiments. Limbus anatomy was found to be quite similar to that of humans and the basic human canaloplasty surgical technique needed little modification aside from the

miniaturization of the catheters. In our experiments, although 150- and 175- μ m catheters were both available, we generally chose to use the 175 μ m. Both catheters have LED lights but the 175- μ m version has the light as a fixed, integral part of the catheter while in the 150- μ m version the light source is a separate piece inside the catheter and has to be removed before injections are made. With the endoscopic camera, the catheter was visualized easily, so in future monkey canaloplasty experiments, it might not be necessary to use catheters with LED lights. These experiments demonstrate that the cynomolgus eye is suitable for SC surgery and gene therapy studies using SC as the delivery site.

In the current study, initial experiments were performed to verify SC, test different catheter configurations, and determine how many clock hours of cannulation could be achieved with the microcatheters. Trypan blue experiments were conducted to see how many clock hours the dye would travel after cannulation. All of these are key issues to address before experiments that inject vector/transgene into SC can be undertaken.

The observations made from the trypan blue experiments lead us to think that the microcatheter may not need to be inserted around the entire circumference of SC to effectively deliver viral vector constructs throughout SC. This would be advantageous because the microcatheters used in canaloplasty could potentially cause trauma to the walls of the SC that might in turn affect the vector/transgene expression in the TM/SC region. Catheterization might also cause microruptures in the inner wall of the SC that could lead to vector/transgene from the canal reaching the AC, although it has yet to be determined whether the resulting low viral titer in the AC would result in clinically relevant transgene expression in surrounding tissues, for example, corneal endothelium or lens epithelium. Although Grieshaber et al.³ demonstrated different grades of fluorescein suffusion from SC to AC, suggesting that the pressure gradient between the 2 compartments might be reversed by controlled paracentesis, we did not do the relevant experiments to allow further comment. As a surgical methods article, this is very much a work in progress.

According to our observations, insertion of the microcatheter into each of the open SC ostia for only 1 clock hour to inject the vector/transgene might also be the easiest and least time consuming, as well as the safest approach, compared to circumferential cannulation. The most effective approach for IOP reduction might be to perform traditional circumferential canaloplasty and suture placement in combination with gene delivery. Long-term effects of this surgical approach should be demonstrated in live-animal experiments. However, in the current study, the animals were donated by the Primate Center; postsurgical evaluation of IOP was not allowed and they had to be sacrificed immediately. Thus, preanesthesia, postanesthesia, and pre-euthanasia IOPs were not measured.

Ocular gene therapy, whether the therapeutic construct is delivered via intracanalicular, intracameral, intravitreal, subretinal, or subconjunctival injection, transgene expression in neighboring tissues is a possibility. Advances in vector design, with improved tropisms for specific ocular tissues, should reduce off-target expression but the question of whether small amounts of expression in the iris, for example, lead to a clinically relevant negative effect for the patient will need to be addressed.

In conclusion, canaloplasty is a promising surgical technique for delivering gene therapeutic constructs designed to decrease outflow resistance in the TM/SC region. It might also be possible to perform both surgical suture and genetic canaloplasty in the same session. Future experiments will compare vector/transgene expression in the TM/SC region after the 1 clock hour and circumferential microcatheter insertions, determine the effects of cannulation and vector/transgene expression on SC structures and IOP, and monitor the extent and location of GFP transgene expression in the tissues surrounding the AC and its relationship to IOP. The effect of paracentesis on the pressure gradient between SC and AC and its impact on vector/transgene expression in the TM/SC region will also be investigated. These experiments will shed light on the applicability of localized gene therapy in SC and its clinical relevance.

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