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András M. Komáromy
University of Pennsylvania, komaromy@vet.upenn.edu

Signe E. Varner

Eugene de Juan

Gregory M. Acland

Gustavo D. Aguirre
University of Pennsylvania

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Abstract

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Keywords

animal model, dog, retinitis pigmentosa, subretinal injection

Disciplines

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Application of a New Subretinal Injection Device in the Dog

András M. Komáromy,* Signe E. Varner,† Eugene de Juan,‡ Gregory M. Acland,§ and Gustavo D. Aguirre*

*School of Veterinary Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA
†SurModics, Inc., Irvine, CA 92606, USA
‡Beckman Vision Center, University of California, San Francisco, CA 94143, USA
§Baker Institute, Cornell University, Ithaca, NY 14853, USA

The use of a new subretinal injection device (RetinaJect™ Subretinal Cannula, SurModics, Inc., Eden Prairie, MN) to access the subretinal space in the canine model was evaluated. Subretinal injections were performed in 33 mongrel dogs between 2 and 52 months of age (median = 9 months). In 5 normal dogs the injection of 150 µl saline or India ink occurred by using a conventional subretinal injection device (CSID) with a 30-gauge anterior chamber irrigating cannula. The sclera had to be surgically exposed and penetrated before the subretinal injection with the CSID could occur. After removing the CSID, the conjunctiva over the sclerotomy site had to be closed. In a second group of 28 dogs [16 normals, 10 *RPE65* mutants, and 2 with progressive rod cone degeneration (*prcd*)], the 25-gauge needle of the RetinaJect™ was used to penetrate the conjunctiva and the sclera. Once the tip of the needle was close to the retinal surface, a 39-gauge polyimide cannula was extended and brought into apposition with the retina for the subsequent subretinal injection of 150 µl saline, India ink, or adeno-associated virus (AAV). No closure of the conjunctiva was required. The animals were clinically monitored between 1 and 59 weeks after surgery. From this second group 25 eyes were harvested for routine histological analysis either immediately after surgery or after a clinical observation time of between 1 and 40 weeks. Both devices provided equally successful access to the subretinal space. The main advantage of the RetinaJect™ was that no surgical dissection was required; this led to a shorter procedure time and milder postoperative conjunctival swelling. In summary, the use of the RetinaJect™ can be recommended as an alternative to the CSID for subretinal injections in dogs.

Key words: Animal model; Dog; Retinitis pigmentosa; Subretinal injection

INTRODUCTION

Subretinal injections have been used for the application of drugs, cells, and gene therapy vectors. In order to reach photoreceptor or retinal pigment epithelial (RPE) cells with an agent, subretinal application is required (7, 9,13). Successful gene therapy of inherited retinal degenerations by subretinal injection has been described in various animal models, including the Royal College of Surgeons (*RCS*) rat (11,14), the retinal degeneration (*rd*) mouse (6,8), and the retinal degeneration slow (*rds*) mouse (4). Our group, together with other collaborators, has reported the successful gene replacement therapy in a dog model for Leber congenital amaurosis with an *RPE65* mutation (1,2).

Various techniques and devices have been described for subretinal injections (5,13). In the past, our group has used a custom-built injection device with a small-gauge, blunt-tipped cannula for subretinal injections in dogs (5). Recently, we (S.V. and E.dJ.) developed a new device (RetinaJect™ Subretinal Cannula, SurModics, Inc., Eden Prairie, MN) for subretinal injections that permits more efficient and easier access to the subretinal space. The purpose of this study was to evaluate the RetinaJect™ Subretinal Cannula for the subretinal injections in dogs. Outcome measures were successful acute subretinal bleb formation and degree of ocular inflammation during the first week after surgery.

MATERIALS AND METHODS

Animals and Anesthesia

All procedures in this study were approved by the Institutional Animal Care and Use Committee of the University of Pennsylvania, and were done in accordance with the ARVO Statement for the Use of Animals

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Address correspondence to András M. Komáromy, D.M.V., Ph.D., Department of Clinical Studies, School of Veterinary Medicine, University of Pennsylvania, 3900 Delancey Street, Philadelphia, PA 19104, USA. Tel: 215-573-2695; Fax: 215-573-2162; E-mail: komaromy@vet.upenn.edu

in Ophthalmic and Vision Research. Mongrel dogs between 2 and 52 months of age (median = 9 months) were used (Tables 1 and 2). Of the 33 dogs, 21 animals were normal (2 with few retinal folds), 10 dogs were *RPE65* mutants, and 2 dogs were affected by progressive rod-cone degeneration (*prcd*) (3) (Tables 1 and 2). The animals were injected subretinally in either one or both eyes. Some of the unilaterally injected dogs were injected in the contralateral eye 1 week later (Tables 1 and 2).

Topical anti-inflammatory premedication consisted of prednisolone acetate 1% and flurbiprofen 0.03% administered every 30 min during the 2-h time period before surgery. Mydriasis was achieved with topical tropicamide 1%, phenylephrine 10%, and atropine 1%. For survival surgery, systemic antibiotics (amoxicillin 10 mg/kg SQ q12 h) and nonsteroidal anti-inflammatories (carprofen 2 mg/kg PO q12 h) were administered before and continued for 2 days after surgery.

The dogs were premedicated for anesthesia with atropine sulfate (0.02–0.04 mg/kg SQ) and acepromazine maleate (0.5 mg/kg SQ). Anesthesia was induced with IV thiopental sodium (20–30 mg/kg). The dogs were intubated, and inhalation anesthesia was maintained with a gas mixture of isoflurane and oxygen on a semiclosed system. Intravenous fluid (0.9% sodium chloride, 10 ml/kg/h) was administered during the surgery.

Preparation of Eye for Subretinal Injection

The globe was positioned by retrobulbar injection of 0.9% sodium chloride (volume to effect), and by nylon

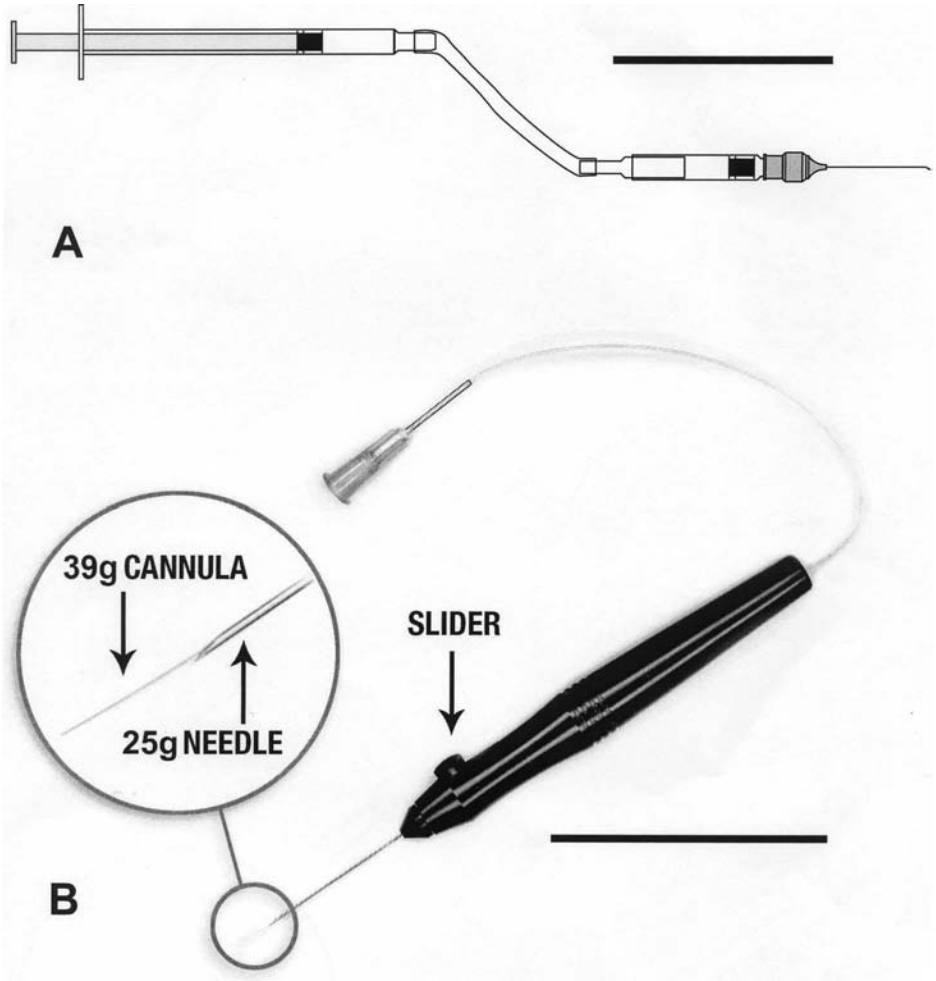

Figure 1. Subretinal injection devices. (A) The conventional subretinal injection device (CSID) consists of a 30-gauge Knolle anterior chamber irrigating cannula connected to a modified plastic tuberculin syringe, which contains the reagent to be injected and a rubber plunger. This syringe is connected through flexible extension tubing to a second tuberculin syringe containing 0.9% sodium chloride (saline). (B) A slider on the RetinaJect™ moves a 39-gauge polyimide cannula within a 25-gauge needle. A syringe with reagent can be connected to the needle hub at the end of the flexible silicone tubing. Scale bar: 5 cm.

Table 1. Summary of Subretinal Injections With the CSID

Dog	Eye	Age (Months)	Sex	Weight (kg)	Preexisting Retinal Disease Status	Agent	Postoperative Observation: Clinical (Weeks)	Postoperative Observation: Histology
L25	right	2	M	6	none	saline	0	Y
L25	left					saline	0	Y
L26	right	2	M	6	none	saline	1	Y
L29	right	2	M	6	none	India ink	0	Y
L29	left					India ink	0	Y*
BR226	right	4	M	9	none	India ink	0	Y
BR226	left					India ink	0	Y
BR233	right	7	F	13	none	India ink	1	Y
BR233	left					saline	0	Y

*Sub-RPE injection.

stay suture (5-0 Ethilon®, Ethicon, Somerville, NJ) placed in the ventral sclera next to the limbus. The eye was aseptically prepared with 0.5% diluted povidone-iodine solution, and topical proparacaine 0.5% was applied for additional analgesia. In order to improve access to the globe, the palpebral fissure was kept open with a lid speculum, and in smaller dogs, a lateral canthotomy had to be performed for better access. A surgical microscope was brought in position and used for the entire surgery. Aqueous paracentesis with a 30-gauge needle was done at the limbus, and 150 µl of aqueous humor removed. An ocular Machemer magnifying vitrectomy lens (OMVI; Ocular Instruments, Inc., Bellevue, WA) placed on the cornea was used to visualize the retina for the subretinal injections.

Conventional Subretinal Injection Device (CSID)

The CSID is shown in Figure 1A. A 30-gauge Knolle anterior chamber irrigating cannula was connected to a modified plastic tuberculin syringe containing the reagent to be injected and a rubber plunger. This syringe was connected through flexible extension tubing to a second tuberculin syringe containing 0.9% sodium chloride (saline) and operated by an assistant. The lateral sclera was exposed, by dissecting the overlying conjunctiva and Tenon's capsule, and focally cauterized. Both sclera and pars plana were penetrated perpendicularly with a 25-gauge needle, 6 mm posterior to the limbus (10). The needle was removed and the 30-gauge cannula of the CSID was inserted through the sclerotomy site into the vitreous. The blunt tip of the cannula was tangentially brought into contact with the retina, and 150 µl of either saline or India ink was briskly injected (Table 1). India ink was filtered through a 0.45-µm minipore filter before use. The CSID was removed and the conjunctiva closed in a simple continuous pattern with polyglactin 910 suture (7-0 Vicryl®, Ethicon). When performed, the lateral canthotomy was closed with nylon suture (5-0 Ethilon®).

RetinaJect™ Subretinal Cannula

The RetinaJect™ is comprised of a 25-gauge needle for penetrating the conjunctiva and sclera (Fig. 1B). This needle is mounted on a hand piece and contains a 39-gauge polyimide cannula mounted in larger silicone tubing. The polyimide cannula is attached to a slider within the handpiece, allowing it to be advanced through the 25-gauge needle. A Luer adapter is located at the end of the silicone tubing and allows the connection of a syringe or other infusion device (Fig. 1B).

For injection, dissection of the conjunctiva and sclera was not required. The globe was penetrated with the 25-gauge needle of the RetinaJect™ 6 mm posterior to limbus, and the tip of the needle was brought into proximity of the retinal surface. Using the slider in the hand piece, the 39-gauge cannula of the RetinaJect™ was extended from the tip of the 25-gauge needle and brought tangentially into contact with the retina followed by the brisk injection of 150 µl of saline, India ink, or adeno-associated virus (AAV) (Table 2). The RetinaJect™ was removed. Except for the lateral canthotomy (if performed) no closure was necessary.

Postoperative Management

Some dogs were euthanized immediately at the conclusion of surgery, and others were recovered and observed for 1 week or longer after the subretinal injection. At 1 week some of the unilaterally injected dogs were

Table 2. Summary of Subretinal Injections With the RetinaJect™

Dog	Eye	Age (Months)	Sex	Weight (kg)	Preexisting Retinal Disease Status	Agent	Postoperative Observation: Clinical (Weeks)	Postoperative Observation: Histology
L30	right	5	F	13	none	India ink	1	Y
L30	left					saline	0	Y
L23	right	2	M	6	none	saline	1	Y
L23	left					India ink	0	Y*
EM125	right	3	M	11	none	saline	0	Y
EM125	left					saline	0	Y
EM127	right	3	M	10	none	saline	0	Y
EM127	left					saline	0	Y
EM128	right	3	F	11	none	saline	0	Y
EM128	left					saline	0	Y
GS45	right	15	M	20	none	AAV5	5	Y
GS45	left					AAV5	5	Y
GS46	right	5	M	15	none	AAV5	8	Y
GS46	left					AAV5	8	Y
GS47	right	5	M	13	none	AAV5	8	Y
GS47	left					AAV5	8	Y
GS54	right	4	M	9	none	AAV5	8	Y
GS54	left					AAV5	8	Y
GS55	right	4	M	8	none	AAV5	8	Y
GS55	left					AAV5	8	Y
GS60	right	4	F	8	none	AAV5	2	Y
GS60	left					AAV5	2	Y
BR213	right	28	F	17	*RPE65*	AAV2	40	Y
BR213	left				*RPE65*	AAV2	40	Y
BR225	right	21	M	16	*RPE65*	AAV2	40	Y
D285	right	16	M	20	retinal folds	saline	7	N
D295	right	9	M	20	retinal folds	saline	7	N
N237	left	11	M	9	none	saline	7	N
N233	left	12	F	9	none	saline	7	N
N241	right	11	F	9	none	saline	7	N
X192	right	38	M	12	*prcd*	saline	59	N
X197	right	37	F	9	*prcd*	saline	59	N
BR210	right	29	F	23	*RPE65*	AAV2	44	N
BR210	left				*RPE65*	AAV2	44	N
EMB14	right	23	M	23	*RPE65*	AAV2	44	N
EMB14	left				*RPE65*	AAV2	44	N
EMB15	right	23	F	18	*RPE65*	AAV2	44	N
BR230	right	21	F	18	*RPE65*	AAV2	44	N

Table 2. Continued

Dog	Eye	Age (Months)	Sex	Weight (kg)	Preexisting Retinal Disease Status	Agent	Postoperative Observation	
							Clinical (Weeks)	Histology
BR57	right	52	F	20	*RPE65*	AAV2	44	N
BR57	left				*RPE65*	AAV2	44	N
EMB62	right	9	F	16	*RPE65*	AAV2	6	N
EMB62	left				*RPE65*	AAV2	6	N
EMB59	right	9	F	18	*RPE65*	AAV2	6	N
EMB59	left				*RPE65*	AAV2	6	N
BR303	right	7	F	14	*RPE65*	AAV2	6	N
BR303	left				*RPE65*	AAV2	6	N

prcd: progressive rod-cone degeneration; *RPE65*: *RPE65* mutant (Leber congenital amaurosis); AAV2, AAV5: adeno-associated virus type 2 and 5.
*Sub-RPE injection.

anesthetized again for subretinal injection of the contralateral eye and euthanized following the second procedure (Tables 1 and 2).

In the survival surgery, 5 mg of triamcinolone acetonide was injected subconjunctivally before recovery from anesthesia, and an antibiotic-steroid ointment (Neomycin-Polymyxin B-Dexamethasone 0.1% ointment) was used topically. The postoperative medical treatment included the continuation of both systemic amoxicillin and carprofen for 2 days (same dosages as before surgery), and topical prednisolone acetate 1% and atropine 1% were used for 1 week as needed to control mild postoperative uveitis.

Histologic Evaluation

In addition to the clinical evaluation, histologic studies were performed in 34 eyes of 18 dogs (Tables 1 and

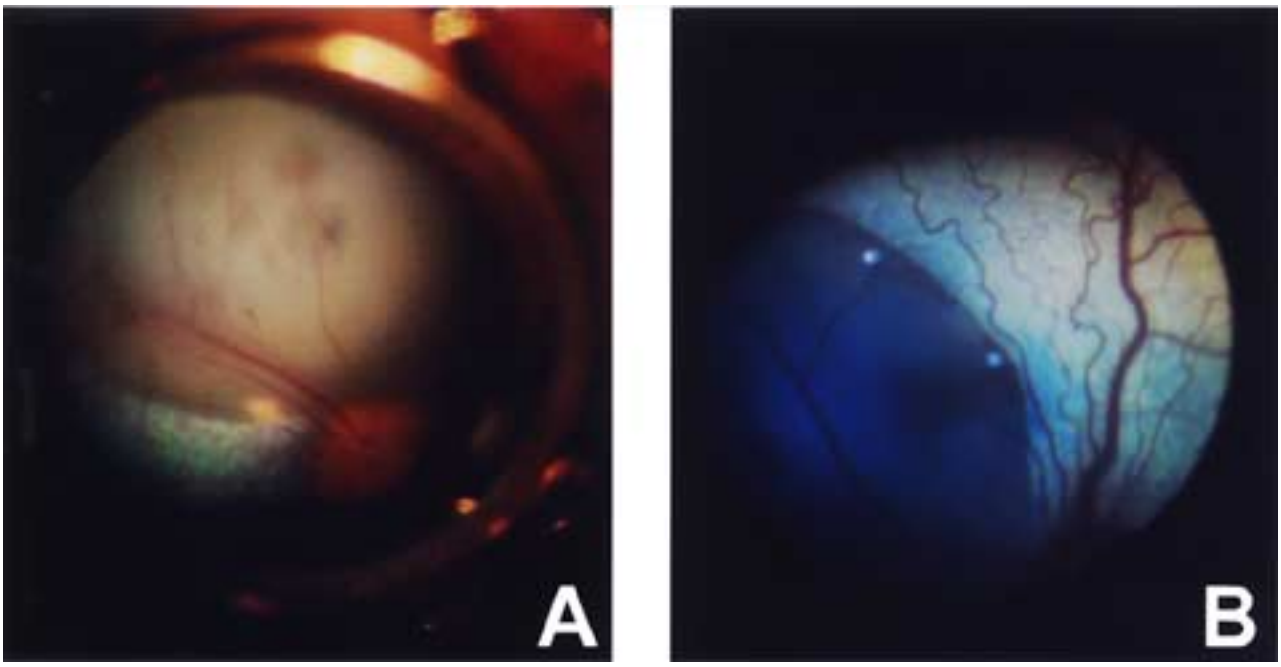

Figure 2. Subretinal blebs as seen through the surgical microscope and the Machemer lens (A), or by indirect ophthalmoscopy (B) immediately after the injection with the RetinaJect™. The green reflection represents the tapetal fundus.

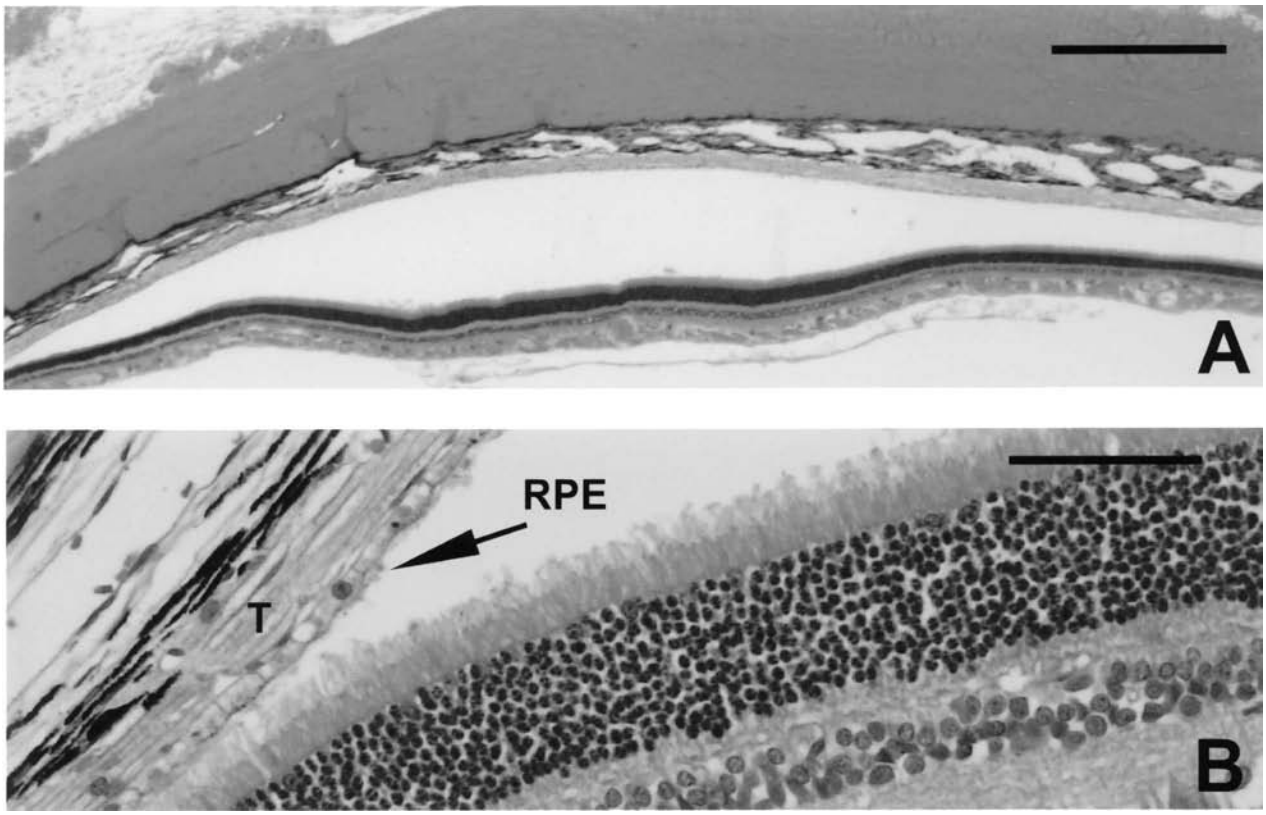

Figure 3. Subretinal bleb seen over the tapetum (T) immediately after saline injection (A, B). Scale bar: 500 μm (A) and 100 μm (B) [original magnification ×20 (A) and ×200 (B)].

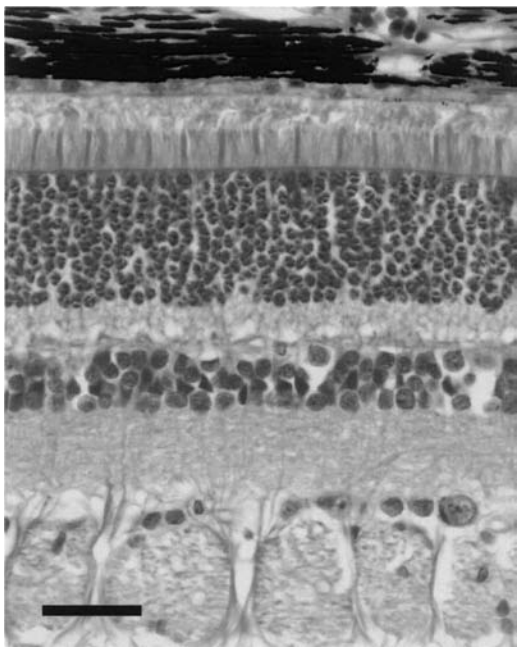

Figure 4. The retina was reattached and without any obvious pathologic changes 1 week after the subretinal injection of saline. Scale bar: 50 μm (original magnification ×400).

2). These dogs were euthanized with an IV overdose of sodium pentobarbital. The eyes were enucleated and fixed either in Bouin solution for paraffin embedding or in 4% paraformaldehyde solution for embedding in Optimal Cutting Temperature (OCT) compound. The globes were processed routinely and sectioned through the subretinal injection areas. Paraffin sections were stained with hematoxylin and eosin (H&E).

RESULTS

A total of 9 eyes in 5 dogs were injected with the CSID while 46 eyes in 28 dogs were injected with the RetinaJect™. All 5 dogs treated with the CSID were normal and were between 2 and 7 months of age (median = 2 months) (Table 1). Using the CSID, 4 eyes were injected with saline and 5 with India ink. Of these 9 injected eyes, 2 were followed for 1 week and 7 were harvested immediately after the subretinal injection (Table 1). Because the 100% success rate matched our previous experience on 29 eyes (1), no additional dogs were injected with the CSID.

Of the 28 dogs injected with the RetinaJect™, 16 animals were considered normal (2 with few retinal folds), 10 were *RPE65* mutants, and 2 were affected by *prcd*.

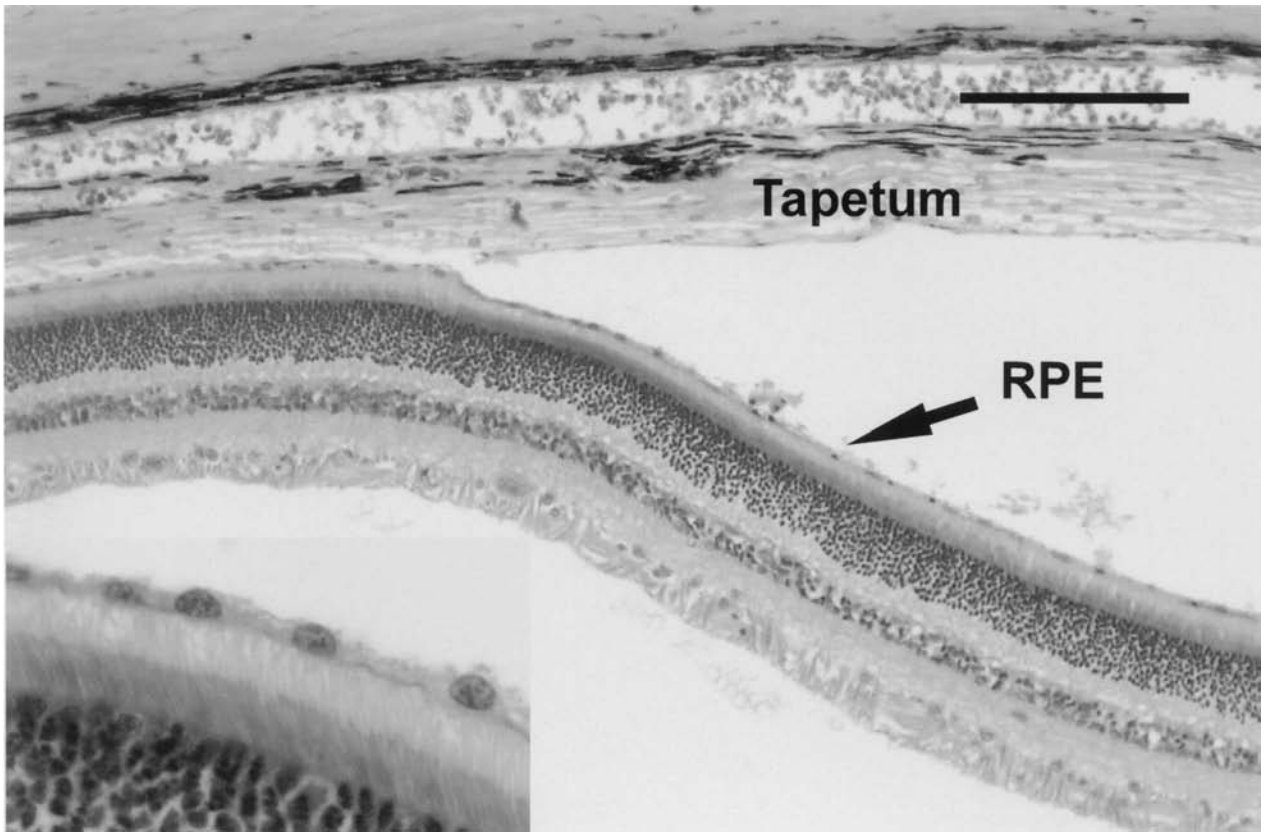

Figure 5. In two cases the bleb occurred between the RPE and the tapetum/choroid. The inset shows magnification of the detached RPE layer. Scale bar: 200 µm (original magnification ×100).

The dogs of the RetinaJect™ group were between 2 and 52 months of age (median = 10 months) (Tab. 2). Using the RetinaJect™, saline was injected in 15 eyes, India ink in 2 eyes, and AAV in 29 eyes. Of these 46 injected eyes, 8 were collected immediately after surgery, 2 each were removed at 1 and 2 weeks postinjection, and the remaining 34 eyes were monitored clinically between 5 and 59 weeks (Table 2). Of these latter eyes, 2 were collected after 5 weeks, 8 after 8 weeks, and 3 after 40 weeks; the remaining eyes are still being monitored clinically as part of a separate study (Table 2). Except for the technical outcome measures of this study, the results of the AAV injections will be reported elsewhere.

Comparison of RetinaJect™ With CSID

The success rate of the subretinal injections was the same with both devices. Subretinal blebs were visible in all eyes immediately after the injection (Fig. 2), and no signs of ocular discomfort were observed in any of the operated animals. While chemosis was mild in all eyes after surgery, the conjunctival swelling appeared less with RetinaJect™ than with CSID. Clinical signs of ocular inflammation were mild and similar for the two injection devices. No signs of inflammation were visible 1 week postinjection. Histologically, no difference could be observed between the two techniques (Fig. 3).

While limbal-based conjunctival flaps had to be dissected and then closed in all the eyes in which the CSID was used, no surgical dissection and closure where required for the application of the RetinaJect™. This meant that the surgery time was shorter for the Retina Ject™ compared to the CSID; however, surgery times were not measured quantitatively. The injection of India ink with the RetinaJect™ revealed less leakage from the subretinal bleb into the vitreous than with the CSID. Subjectively, the retinotomy sites appeared smaller after injection with the RetinaJect™ than with the CSID but this could not be confirmed histologically.

Other Findings

The injected subretinal fluid was absorbed and the retinas reattached within 24–48 h. Histologically, the subretinal blebs were only visible if the eyes were harvested immediately after the injection (Fig. 3). One week after the subretinal injection, no signs of subretinal fluid could be seen clinically or histologically (Fig. 4).

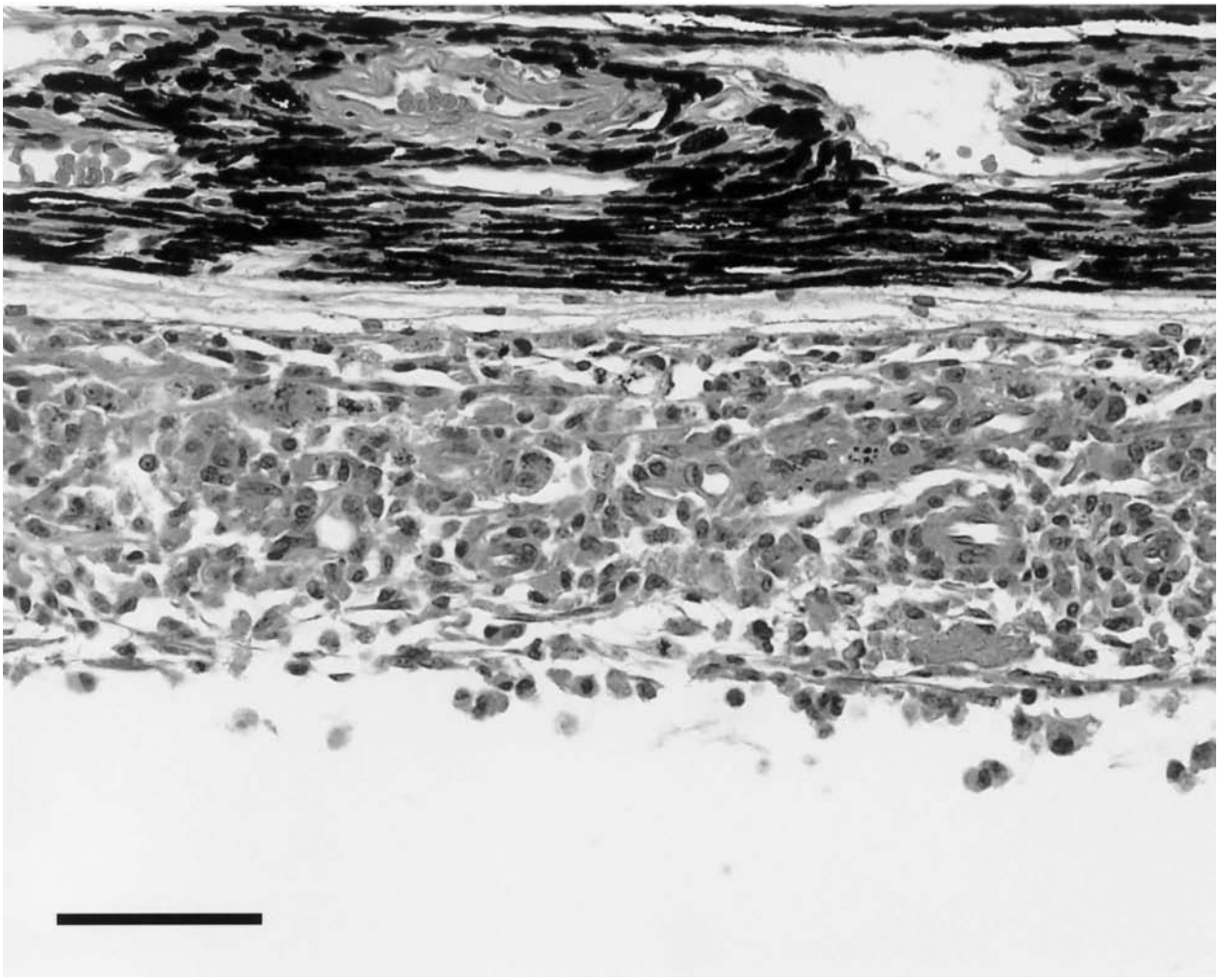

Figure 6. Total retinal degeneration and disorganization 1 week after subretinal injection of India ink. Scale bar: 100 μm (original magnification ×200).

With each injection device sub-RPE injections were observed histologically in one eye (Tables 1 and 2, Fig. 5). Because the injections were placed in the tapetal region, where the RPE lacks pigmentation, the difference between the subretinal and sub-RPE injections could not be appreciated, either during surgery or by ophthalmoscopy after the procedure.

We noted clinical signs indicating loss of retinal function (negative menace response, pupillary light reflexes, and dazzle reflex) 1 day after the injection of India ink. Histologic evaluation confirmed the generalized retinal degeneration (Fig. 6).

DISCUSSION

This study showed the same effectiveness of subretinal injections in dogs using either the RetinaJect™ or the CSID. However, there were advantages when using the RetinaJect™ compared to the CSID. Chief among these is the fact that RetinaJect™ allows combining multiple steps of the surgery. Conjunctiva, Tenon's capsule, sclera, and pars plana were penetrated with the 25-gauge needle, which also protected the sensitive 39-gauge cannula inside. Once the tip of the needle was close to the retinal surface, the 39-gauge cannula could be extended with a slider for the subsequent subretinal injection. This approach was less traumatic, and no closure of surgical wounds was required.

The number of eyes injected with the CSID was smaller than the number injected with the RetinaJect™. Because the CSID was previously successfully used for 29 surgeries on *RPE65* mutant eyes (1), and the results were identical to the ones listed here, we did not consider it necessary to operate more dogs.

New treatment options for inherited retinal degenerations, such as gene therapy, will soon enter clinical trials. For some of these new treatments subretinal injec-

tions will be required. The RetinaJect™ will simplify this approach, and can be used in either normal or moderately degenerate retinas.

Histologically, we found one eye with a sub-RPE bleb for each injection device. This came as a surprise because clinically all the blebs appeared similar. We are not aware of any previous reports that described the incidence of sub-RPE blebs with subretinal injections. This observation could only be made when the eyes were harvested immediately after the injections. Because the subretinal blebs disappeared within 24–48 h after surgery, a sub-RPE bleb could not be seen at a later time. One has to be aware of this phenomenon because it could explain a potential failure of treatment when therapy is directed specifically to the cells that line the interphotoreceptor space. Sub-RPE blebs should be easier recognized in areas of the fundus with a pigmented RPE. This would be the case in the nontapetal part of the canine fundus. Further studies would have to be done to determine the frequency of sub-RPE blebs with subretinal injections using the RetinaJect™ or CSID.

While India ink was very useful to evaluate leakage from the subretinal bleb into the vitreous, its use is not recommended for survival studies. India ink led to a generalized retinal degeneration in our dogs, even in areas away from the subretinal bleb. To the best of our knowledge, retinal toxicity of India ink has not been described previously. One experimental application of India ink is the induction of ocular hypertension in rodents after anterior chamber injection followed by laser treatment of the episcleral vessels (12). There are no indications that India ink causes any retinal degeneration in these animals.

In summary, the use of RetinaJect™, a new subretinal injection device with 25-gauge needle and extendable 39-gauge cannula, can be recommended as an alternative to the CSID for subretinal injections in dogs.

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REFERENCES

1. Acland, G. M.; Aguirre, G. D.; Bennett, J.; Aleman, T. S.; Cideciyan, A. V.; Bennicelli, J.; Dejneka, N. S.; Pearce-Kelling, S. E.; Maguire, A. M.; Palczewski, K.; Hauswirth, W. W.; Jacobson, S. G. Long-term restoration of rod and cone vision by single dose rAAV-mediated gene transfer to the retina in a canine model of childhood blindness. Mol. Ther. 12:1072–1082; 2005.
2. Acland, G. M.; Aguirre, G. D.; Ray, J.; Zhang, Q.; Aleman, T. S.; Cideciyan, A. V.; Pearce-Kelling, S. E.; Anand, V.; Zeng, Y.; Maguire, A. M.; Jacobson, S. G.; Hauswirth, W. W.; Bennett, J. Gene therapy restores vision in a canine model of childhood blindness. Nat. Genet. 28:92–95; 2001.
3. Aguirre, G. D.; Acland, G. M. Variation in retinal degeneration phenotype inherited at the *prcd* locus. Exp. Eye Res. 46:663–687; 1988.
4. Ali, R. R.; Sarra, G. M.; Stephens, C.; Alwis, M. D.; Bainbridge, J. W.; Munro, P. M.; Fauser, S.; Reichel, M. B.; Kinnon, C.; Hunt, D. M.; Bhattacharya, S. S.; Thrasher, A. J. Restoration of photoreceptor ultrastructure and function in retinal degeneration slow mice by gene therapy. Nat. Genet. 25:306–310; 2000.
5. Bennett, J.; Anand, V.; Acland, G. M.; Maguire, A. M. Cross-species comparison of *in vivo* reporter gene expression after recombinant adeno-associated virus-mediated retinal transduction. Methods Enzymol. 316:777–789; 2000.
6. Bennett, J.; Tanabe, T.; Sun, D.; Zeng, Y.; Kjeldbye, H.; Gouras, P.; Maguire, A. M. Photoreceptor cell rescue in retinal degeneration (*rd*) mice by *in vivo* gene therapy. Nat. Med. 2:649–654; 1996.
7. Dudus, L.; Anand, V.; Acland, G. M.; Chen, S. J.; Wilson, J. M.; Fisher, K. J.; Maguire, A. M.; Bennett, J. Persistent transgene product in retina, optic nerve and brain after intraocular injection of rAAV. Vis. Res. 39:2545–2553; 1999.
8. Kumar-Singh, R.; Farber, D. B. Encapsidated adenovirus mini-chromosome-mediated delivery of genes to the retina: application to the rescue of photoreceptor degeneration. Hum. Mol. Genet. 7:1893–1900; 1998.
9. Schraermeyer, U.; Thumann, G.; Luther, T.; Kociok, N.; Armhold, S.; Kruttwig, K.; Andressen, C.; Addicks, K.; Bartz-Schmidt, K. U. Subretinally transplanted embryonic stem cells rescue photoreceptor cells from degeneration in the RCS rats. Cell Transplant. 10:673–680; 2001.
10. Smith, P. J.; Pennea, L.; MacKay, E. O.; Mames, R. N. Identification of sclerotomy sites for posterior segment surgery in the dog. Vet. Comp. Ophthalmol. 7:180–191; 1997.
11. Smith, A. J.; Schlichtenbrede, F. C.; Tschernutter, M.; Bainbridge, J. W.; Thrasher, A. J.; Ali, R. R. AAV-Mediated gene transfer slows photoreceptor loss in the RCS rat model of retinitis pigmentosa. Mol. Ther. 8:188–195; 2003.
12. Ueda, J.; Sawaguchi, S.; Hanyu, T.; Yaoeda, K.; Fukuchi, T.; Abe, H.; Ozawa, H. Experimental glaucoma model in the rat induced by laser trabecular photocoagulation after an intracameral injection of India ink. Jpn. J. Ophthalmol. 42:337–344; 1998.
13. Verdugo, M. E.; Alling, J.; Lazar, E. S.; del Cerro, M.; Ray, J.; Aguirre, G. Posterior segment approach for subretinal transplantation of injection in the canine model. Cell Transplant. 10:317–327; 2001.
14. Vollrath, D.; Feng, W.; Duncan, J. L.; Yasumura, D.; D'Cruz, P. M.; Chappelow, A.; Matthes, M. T.; Kay, M. A.; LaVail, M. M. Correction of the retinal dystrophy phenotype of the RCS rat by viral gene transfer of *Mertk*. Proc. Natl. Acad. Sci. USA 98:12584–12589; 2001.