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Original Article

Investigation of the maximum feasible volume of subretinal injections into rat and cynomolgus monkey eyes

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ABSTRACT — Subretinal injection is widely used in gene therapy research on age-related macular degeneration and retinitis pigmentosa. We investigated a method of injection and the maximum feasible volume for subretinal injections in rats and monkeys to support future studies for evaluation of new therapies in these animals. Physiological saline was injected subretinally into the eyes of male adult rats (n = 3 eyes/group) and cynomolgus monkeys (n = 3 eyes/group). The eyes were then examined by ophthalmoscopy (slit lamp and ocular fundus examinations), optical coherence tomography (OCT), intraocular pressure (IOP) measurements, or electroretinography (ERG). Rats received physiological saline at 1, 2, 5 µL/eye (2 sec/eye, bolus) and 5 µL/eye (5 µL/min). Monkeys received physiological saline at 50, 100, 150, 200, and 250 µL/eye (10 µL/sec). In all rats and cynomolgus monkeys, successful injection was visually confirmed by bleb formation using OCT at the appropriate sites immediately after injection. IOP increased more than 2-fold after injection of 5 µL/eye (5 µL/2 sec) compared with before injection but remained virtually unchanged at other volumes. Monkeys that received 200 or 250 µL/eye showed a marked increase in IOP immediately after injection, with functional abnormality observed in ERG. In conclusion, bleb formation at the appropriate sites was confirmed by OCT, demonstrating successful subretinal injections to rat and monkey eyes. When physiological saline solution is subretinally injected, the maximum feasible volume is considered to be 5 µL/eye (5 µL/min) in rats and 150 µL/eye (10 µL/sec) in cynomolgus monkeys, based on IOP and ERG results.

Key words: Subretinal injection (SR), Bleb formation, Optical coherence tomography (OCT), Intraocular pressure (IOP), Electroretinography (ERG), Cynomolgus monkey, Rat

INTRODUCTION

In recent years, research on gene therapy in the field of ophthalmology is becoming more active, and gene therapy for hereditary age-related macular degeneration and retinitis pigmentosa in particular (which can cause vision loss) is expected to become a practical advanced therapy (Russell *et al*., 2017; Bordet and Behar-Cohen, 2019; Ziccardi *et al*., 2019; Akyol and Lotery, 2020; Amato *et al*., 2021; Chiu *et al*., 2021; Georgiou *et al*., 2021; Guimaraes *et al*., 2021; Tomita and Sugano, 2021). Subretinal injection is a commonly used method for the injection of therapeutic agents between the retinal photoreceptor layer (rod, cone) and pigment epithelial cells (RPE) (Fig. 1). This approach has become the preferred option for the delivery of drugs for posterior eye diseases, showing particular promise in gene delivery to photoreceptors and retinal RPE (Xue *et al*., 2017; Khabou *et al*., 2018; Bertin *et al*., 2020). In addition, subretinal injection has also been used in stem cell therapy in the posterior eye using embryonic stem cells and induced pluripotent stem cells (Bobba *et al*., 2018; Akyol and Lotery, 2020; Wang

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et al., 2020; Miotti *et al*., 2021). Based on the vitreous volume, the appropriate intraocular volume is said to be 1 to 2 µL/eye in rats, and 50 to 100 µL/eye in monkeys (Laude *et al*., 2010; Atsumi *et al*., 2013). However, in gene and cell therapies, larger dose volumes are sometimes required depending on solubility and efficacious dose volume, especially in toxicity studies where high dose volumes are required (Bordet and Behar-Cohen, 2019). Therefore, the establishment of maximum feasible injection volumes and injection procedures are critical to proper evaluation of drug efficacy and toxicity in these experimental animal species. Using optical coherence tomography (OCT), it is possible to investigate the success of subretinal injections and to chronologically monitor the blebs formed at the site of subretinal injection. Using scanning laser ophthalmoscopy (SLO) images, it is further possible to measure the size of blebs. The intraocular pressure (IOP) should be checked after injection, as increased IOP may affect visual function. In addition, electroretinography (ERG) can reveal changes in retinal function. Thus, IOP and ERG are good indicators of possible adverse effects that subretinal injections could have on the eye.

Here, we investigated the maximum feasible subretinal injection volumes for rats and cynomolgus monkeys using physiological saline. Bleb formation after injection was confirmed in the retina by determining the size and position in ocular fundus by OCT examinations, including SLO examinations, followed by IOP and ERG measurements to investigate the impact on the eye.

MATERIALS AND METHODS

Animals

Twelve Sprague-Dawley (Crl:CD[SD]) rats (male, 8-23 weeks of age, Charles River Laboratories Japan, Inc.) and 15 cynomolgus monkeys (male, 4-6 years of age, Cambodia origin) were used in this study. All animals were maintained in accordance with the ethics criteria contained in the bylaws of Shin Nippon Biomedical Laboratories, Ltd., Drug Safety Research Laboratories (SNBL DSR), which is accredited by AAALAC International. The study was approved by the SNBL DSR Institutional Animal Care and Use Committee and conducted in accordance with the statement from the Association for Research in Vision and Ophthalmology.

Environmental conditions were as follows: temperature: 19-25°C (rats) and 23-29°C (cynomolgus monkeys), humidity: 30-70%, ventilation: 15 times/hr, illumination: 12 hr/day of artificial light (7:00-19:00). Rats were housed with 2 animals/cage (cage size: 290 mm [D] x 380 mm [W] x 200 mm [H]) and cynomolgus monkeys were housed with 1 animal/cage (cage size: 680 mm [D] x 620 mm [W] x 770 mm [H]).

Subretinal injections in rats and monkeys

Rats

A topical antibiotic agent (Cravit Ophthalmic Solution 0.5%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) was instilled into the eye once on the day before injection and for 3 consecutive days after injection (including the day of injection). After induction of mydriasis by instillation of an ophthalmic solution combining 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P Ophthalmic Solution, Santen Pharmaceutical Co., Ltd., Osaka, Japan), animals were anesthetized by intramuscular injection of 3.2 mL/kg of a 1:3:4:8 mixture of medetomidine hydrochloride (Domitor, Orion Corporation, Espoo, Finland, 1 mg/mL), midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan, 5 mg/mL), butorphanoltartrate (Vetorphale, Meiji Seika Pharma Co., Ltd., Tokyo, Japan, 5 mg/mL), and physiological saline (Otsuka Pharmaceutical Factory, Tokushima, Japan). The eyelids and the area surrounding the eye were disinfected, and a surface anesthetic for ophthalmology (Benoxil Ophthalmic Solution 0.4%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) was instilled into the eye for injection using a surgical microscope.

Physiological saline (Otsuka Pharmaceutical Factory, Tokushima, Japan) was injected into the subretinal space of the right eye through a 33-gauge needle (Hamilton, Reno, NV, USA). The injection volumes were 1, 2, and 5 µL/eye (2 sec/eye, bolus) and 5 µL/eye (5 µL/min) (n = 3 eyes/group, Table 1). The needle was kept in the bleb for several seconds after the subretinal injection to prevent efflux of the injection solution into the vitreous.

Monkeys

A topical antibiotic agent (Cravit Ophthalmic Solution 0.5%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) was instilled into the eye once on the day before injection and for 3 consecutive days after the day of injection (including the day of injection). After mydriasis induction by instillation of an ophthalmic solution combining 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P Ophthalmic Solution, Santen Pharmaceutical Co., Ltd., Osaka, Japan) and instillation of 0.5% phenylephrine hydrochloride (Neocinedine, Kowa Company, Ltd., Tokyo, Japan), animals were anesthetized by an intramuscular injection with medetomidine hydrochloride (Domitor, Orion Corporation, Espoo, Finland,

Table 1. Injection design and timeline of examinations in rats are shown.

Injection solution	Injection volume (μL/eye)	Injection speed	Number of rats (eyes)	Timeline of examination	
				Pre-injection	After injection
physiological saline	1	2 sec/eye, bolus	3	OP	OP
	2	2 sec/eye, bolus	3	IOP	Photo
	5	2 sec/eye, bolus	3		OCT
	5	5 μL/min	3		IOP

OP: ophthalmoscopy (slit lamp and ocular fundus examinations), Photo: Fundus photo, OCT: optical coherence tomography, and IOP: intraocular pressure measurement.

0.04 mg/kg), midazolam (Dormicum, Astellas Pharma Inc., Tokyo, Japan, 0.3 mg/kg), and butorphanoltartrate (Vetorphale, Meiji Seika Pharma Co., Ltd., Tokyo, Japan, 0.4 mg/kg). The eyelids and the area surrounding the eye were disinfected, and a surface anesthetic for ophthalmology (Benoxil Ophthalmic Solution 0.4%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) was instilled into the eye for injection.

After making a transscleral incision using a 25-gauge trocar (Mani, Inc., Ibaraki, Japan), physiological saline (Otsuka Pharmaceutical Factory, Tokushima, Japan) was injected into the subretinal space through a syringe with a 38-gauge needle (Dutch Ophthalmic Research Center, VC Zuidland, Netherlands). Physiological saline at 50, 100, 150, 200, and 250 μL/eye was subretinally injected into the left eye of 3 animals per injection volume (Table 2) at 10 μL/sec using a surgical microscope. Because larger injection volumes may cause severe adverse events, initially only 1 animal per injection volume underwent subretinal injection, and these animals were euthanized without awakening from anesthesia after examination of the injection sites to confirm bleb formation. After examination, injection was performed for 2 additional animals per injection volume on another day. The needle tip was kept in the bleb for several seconds after the subretinal injection to prevent efflux of the injection solution into the vitreous. Animals to be awakened after the end of the injection were given an intramuscular injection of atipamezole (0.72 mL/kg; Antisedan, Orion Corporation Espoo, Finland, 5.0 mg/mL).

Slit lamp, ocular fundus examinations, fundus photographs, and OCT examinations in rats and monkeys

Slit lamp, ocular fundus examinations, fundus photographs, and OCT examination were conducted for all rats and monkeys. After instillation of an ophthalmic solution combining 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P Ophthalmic Solution, Santen Pharmaceutical Co., Ltd., Osaka, Japan), slit lamp biomicroscope (SL-15 and SL-17, Kowa Company, Ltd., Aichi, Japan) and indirect ophthalmoscope (IO-αSmall Pupil, Neitz Instruments Co., Ltd., Tokyo, Japan) examinations were conducted. Fundus photography was conducted using a Genesis-Df or VX-10α fundus camera (Kowa Company, Ltd., Aichi, Japan). Animals were anesthetized by a 10 mg/kg of intramuscular injection of ketamine hydrochloride (Ketalar for Intramuscular Injection 500 mg, Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan, 50 mg/mL), and OCT images (Heidelberg Spectralis OCT or OCT2, Heidelberg Engineering GmbH, Heidelberg, Germany) were captured. Built-in Spectralis mapping software (Heidelberg Eye Explorer, software version 6.12.3) was used for bleb diameter analysis.

IOP measurements in rats and monkeys

IOP was examined for all animals. The IOP (TonoVet tonometer TV01 or TonoLab tonometer TV02, Tiolat Oy, Finland) was measured before and after subretinal injection while animals were anesthetized for injection.

Full-field ERG measurements

The following tests were performed on 8 monkeys (Table 2):

After the animals were adapted to the darkness for at least 30 min, they were anesthetized by an intramuscular injection of 0.2 mL/kg of a 1:7 mixture of xylazine hydrochloride (Selactar, Bayer Yakuhin Ltd., Osaka, Japan, 20 mg/mL) and ketamine hydrochloride (Ketalar for Intramuscular Injection 500 mg, Daiichi Sankyo Propharma Co., Ltd., Tokyo, Japan, 50 mg/mL). After ocular instillation of an ophthalmic solution combining 0.5% tropicamide and 0.5% phenylephrine hydrochloride (Mydrin-P Ophthalmic Solution, Santen Pharmaceutical Co., Ltd., Osaka, Japan), mydriasis was confirmed, and oxybuprocaine hydrochloride (Benoxyl Ophthalmic Solution 0.4%, Santen Pharmaceutical Co., Ltd., Osaka, Japan) and a combined solution of

Table 2. Injection design and timeline of examinations in cynomolgus monkeys are shown. Injection days 1 and 2 were different days.

Injection day	Injection solution	Injection volume (μL/eye)	Injection speed (μL/sec)	Number of monkeys (eyes)	Timeline of examinations		
					Pre- Injection	After Injection	The day following injection
1	physiological saline	50	10	1	OP	OP Photo OCT	—
		100		1	OP IOP	OP Photo OCT IOP	
		150		1			
		200		1			
		250		1			
2	physiological saline	50	10	2	OP	OP Photo OCT	OP OCT
		100		2	OP IOP ERG	OP Photo OCT IOP ERG	
		150		2			
		200		2			
		250		2			

OP: ophthalmoscopy (slit lamp and ocular fundus examinations), Photo: Fundus photo, OCT: optical coherence tomography, IOP: intraocular pressure measurement, ERG: electroretinography, and —: not examined.

hydroxyethyl cellulose, boric acid, and inorganic salt (Scopisol Solution for Eye, Senju Pharmaceutical Co., Ltd., Osaka, Japan) were instilled into the eye. A contact lens-type electrode (Kyoto Contact Lens Ltd., Kyoto, Japan) was attached to the cornea. After the electric potential stabilized, ERG examination was conducted using an electroretinoscope (ganzfeld system, SG-2002, LKC Technologies, Inc., MD, USA) and an induction reaction recording device (PuREC, PC100-A, Mayo, Ltd., Aichi, Japan).

DA 3 ERG was performed by flashing the flash stimuli (intensity: 0 dB [stimulus flash strength: 3.082 cds/m^2]) once, and the maximal response (3.0 cds/m^2) was measured. The latency and amplitude of the a- and b-waves were analyzed.

Statistical Analysis

The bleb diameters and bleb angles in OCT were expressed as the mean ± standard deviation (SD). The ERG datasets and IOP data underwent a paired t-test at a significance of 5%, and the data were expressed as the mean ± SD for rats and cynomolgus monkeys.

Statistical analyses were conducted using MiTOX (Mitsui Zosen System Research Inc., Kagoshima, Japan) at a significance level of 5%.

RESULTS

Rats

Subretinal injections of physiological saline at 1, 2, and 5 μL/eye (2 sec/eye, bolus) and 5 μL/eye/(5 μL/min) were performed to 3 animals. Bleb formation at the injection site was observed immediately after subretinal injection in both ophthalmoscopic fundus and OCT examinations, confirming that the saline was successfully administered into the subretinal space (Fig. 2). Bleb size did not change during the OCT examination, and there was no evidence of leakage of the administered solution from the subretinal space into the vitreous or out of the eyeball. The IOP increased more than 2-fold after subretinal injection of 5 μL/eye (5 μL/2 sec) when compared with the pre-injection value, while no change in IOP was observed after injection of 1 or 2 μL/eye (2 sec/eye, bolus) or 5 μL/eye (5 μL/min) (Fig. 3).

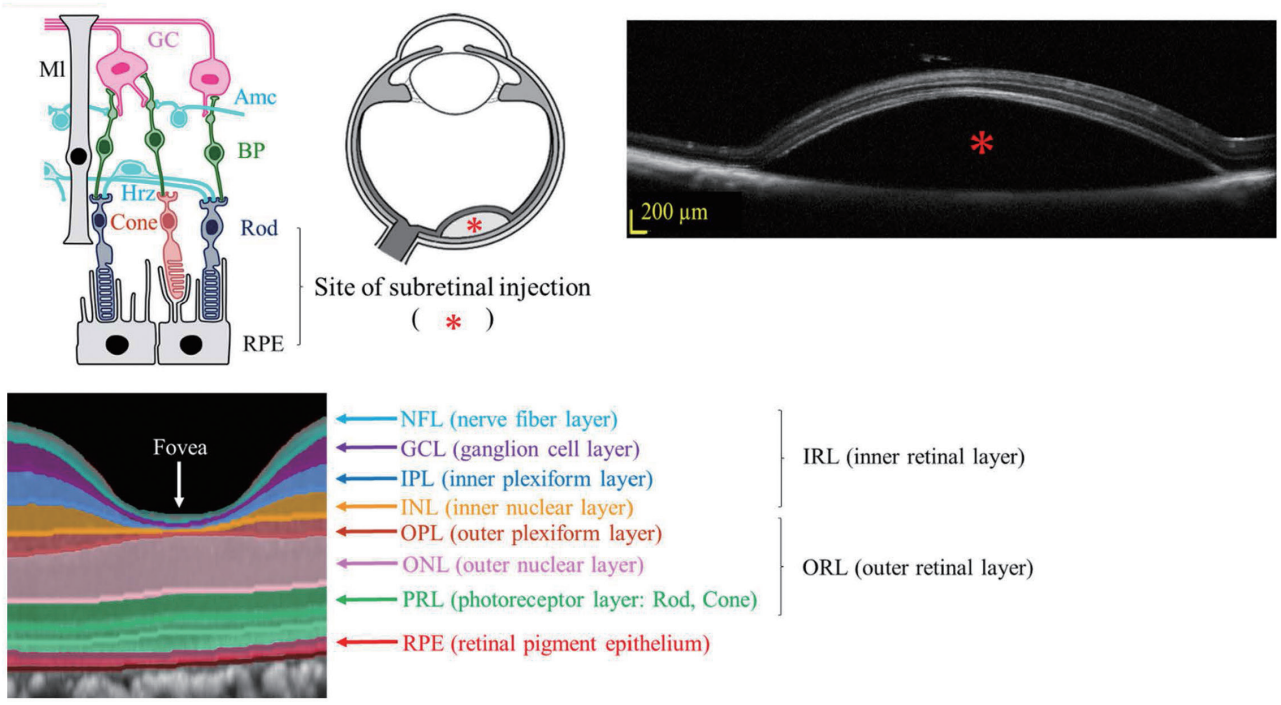

Fig. 1. Schematic drawing of retinal tomogram. MI: Müller cell, GC: ganglion cell, Amc: amacrine cell, BP: bipolar cell, Hrz: horizontal cell and RPE: pigment epithelial cell. Bleb formation at the subretinal injection site of (*). Subretinal injection is a method of injection between the retinal photoreceptor layer (rod, cone) and pigment epithelial cells (RPE).

Cynomolgus monkeys

Subretinal injections of physiological saline at 50, 100, 150, 200, and 250 µL/eye were performed to 3 animals per dose volume (10 µL/sec). As in rats, bleb formation at the injection site was observed immediately after subretinal injection in both ophthalmoscopic fundus and OCT examinations, confirming that the saline was successfully administered into the subretinal space.

In OCT examinations, bleb diameters increased with the injected volumes (Fig. 4). Bleb diameters at 100 µL/eye were 185% those at 50 µL/eye, and from 150 µL/eye, bleb diameters increased less than dose-proportionally: bleb diameters at 150, 200, and 250 µL/eye were 165%, 153%, and 144%, respectively, those at 50 µL/eye.

Detachment between the retinal photoreceptor layer and RPE at the blebs in monkey eyes receiving up to 150 µL/eye mostly disappeared by the day following the subretinal injection in OCT (Fig. 5). With larger injections of 200 and 250 µL, very small interspaces were observed between photoreceptors and the RPE, but these completely disappeared on the second day after subretinal injection (data not shown).

The IOP immediately after subretinal injection of 250 µL/eye was approximately 3-fold higher than the pre-injection value (Fig. 6). At 200 µL/eye, the change in IOP was large, ranging from under half to double the pre-injection value, depending on the individual. At 10 min after injection, the IOP recovered to the pre-injection values in monkeys receiving 200 or 250 µL/eye. There were no IOP changes in monkeys receiving a subretinal injection of 150 µL/eye or less.

DA 3 ERG measurements were performed on the day before and the day after injection, and the amplitude and latency of the a- and b-waves were analyzed in ERG examinations (Fig. 7). Compared with the pre-injection value, the latency of the b-wave was decreased in eyes injected with 250 µL/eye, while the amplitude of the b-wave was attenuated in eyes injected with 200 and 250 µL/eye with a statistically significant decrease.

DISCUSSION

Subretinal injections are widely used in gene therapy research for age-related macular degeneration and retinitis pigmentosa, requiring large volumes to be injected for

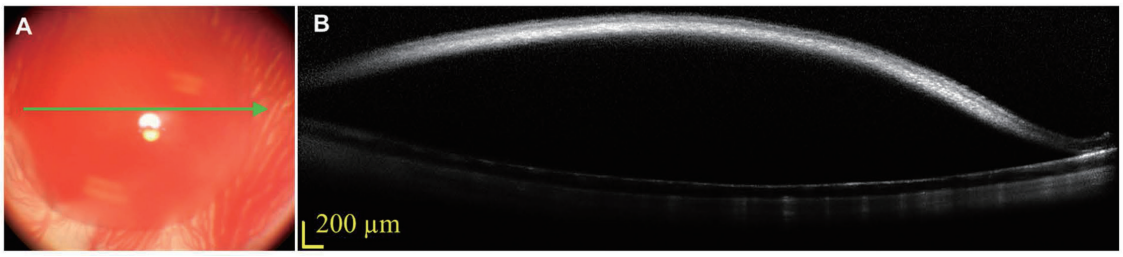

Fig. 2. Fundus photographs and OCT images of subretinal injection sites in rats (A and B). OCT examinations were performed immediately after subretinal injection to confirm that the saline was successfully injected. The green arrow shows the plane for OCT examination.

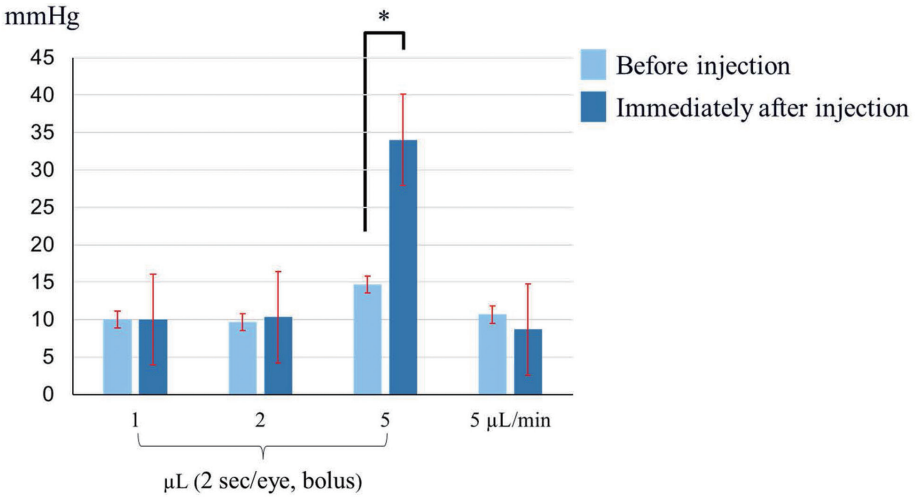

Fig. 3. Intraocular pressure fluctuation after subretinal injection into rat eyes. Subretinal injections were performed with 1, 2, and 5 µL/eye (2 sec/eye, bolus) and 5 µL/eye (5 µL/min), and the IOP was measured using a TonoLab tonometer TV02. Mean ± standard deviation (n = 3 eyes/group). *P < 0.05 (paired t-test).

gene transfer if necessary.

In this study, we investigated the maximum feasible volume of subretinal injection in nonclinical studies.

Rats and cynomolgus monkeys are frequently used in drug safety and efficacy studies, but there are some species-specific differences in the structure of the eye, such as the retinal thickness and vitreous volume (Brock *et al*., 2013; Onodera *et al*., 2015; Shibuya *et al*., 2015). Intraocular injection requires a dose volume that does not cause an excessive increase in IOP. In animal studies, however, there are some cases where a dose volume exceeding that recommended for humans is necessary to properly evaluate any drug's efficacy and safety. Excessively elevated IOP can cause unexpected retinal damage, and therefore the dose volume should be carefully set in animal studies with subretinal injection. In order to properly evaluate the efficacy and safety of the drug, subretinal injection of the defined dose needs to be conducted appropriately, and OCT is a useful tool for capturing the success of subretinal injection and detailed morphologic changes at the injection site. We believe that the ocular characteristics of rats and monkeys should be well understood in order to establish appropriate injection volumes for preclinical evaluation of drug candidates.

In rats, the maximum feasible injection volumes that did not affect IOP were found to be 2 µL/eye when injected for 2 seconds and 5 µL/eye when injected for 1 min. Slowing down the injection speed allowed injection of the largest volume of 5 µL without increasing the IOP.

In monkeys, subretinal injection of 50, 100, 150, 200,

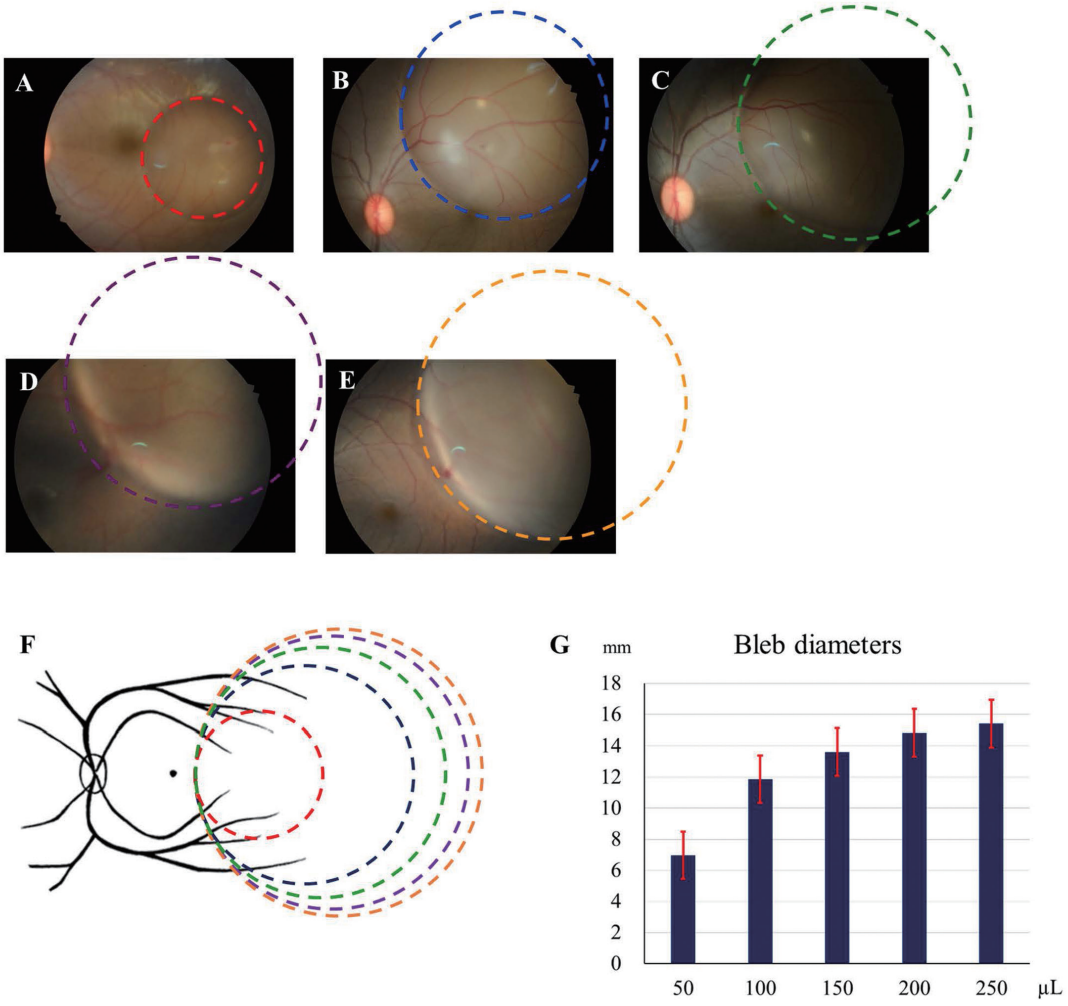

Fig. 4. Fundus photographs of monkey eyes after subretinal injection of different volumes of physiological saline. Bleb diameters in fundus camera indicated in the photos were 6.97 ± 0.55 mm (A), 11.87 ± 0.25 mm (B), 13.60 ± 0.51 mm (C), 14.83 ± 0.41 mm (D), and 15.43 ± 0.51 mm (E) after injection of 50, 100, 150, 200, and 250 µL/eye, respectively (1 representative experiment out of 3 experiments is shown); schematic diagram of the bleb diameter for each injection volume using same colors indicating the bleb diameters in panels A to E; (F) relationship between bleb diameter size and the injection volume (G). Mean ± standard deviation (n = 3 eyes/group).

and 250 µL/eye of physiological saline to left eyes resulted in a dose-dependent increase in bleb size. When subretinally injected at 200 or 250 µL/eye, a space was still observed between the photoreceptor layer and RPE on the day after injection, suggesting that it takes longer time to absorb the injection solution at these volumes. At 100 µL/eye or more, although bleb size increased, the fold increase in bleb diameter was less than the fold increase in injected volume, suggesting that the height of the bleb increased with the volume of injected solution. This increase in bleb height between the photoreceptor layer and RPE, which may have been one of the reasons why the injected solution took longer to be absorbed.

Thinning of the outer nuclear layer (ONL) has been reported in monkeys given a subretinal injection of 200 µL of balanced salt solution with a 41-gauge cannula via a pump system (Ochakovski *et al*., 2017). In the present study, the retinal thickness did not change, partly because the injected solution was physiological saline.

Monkeys showed a marked increase in IOP immediately after injection at 200 and 250 µL/eye although the IOP returned to the pre-injection values within 10 min

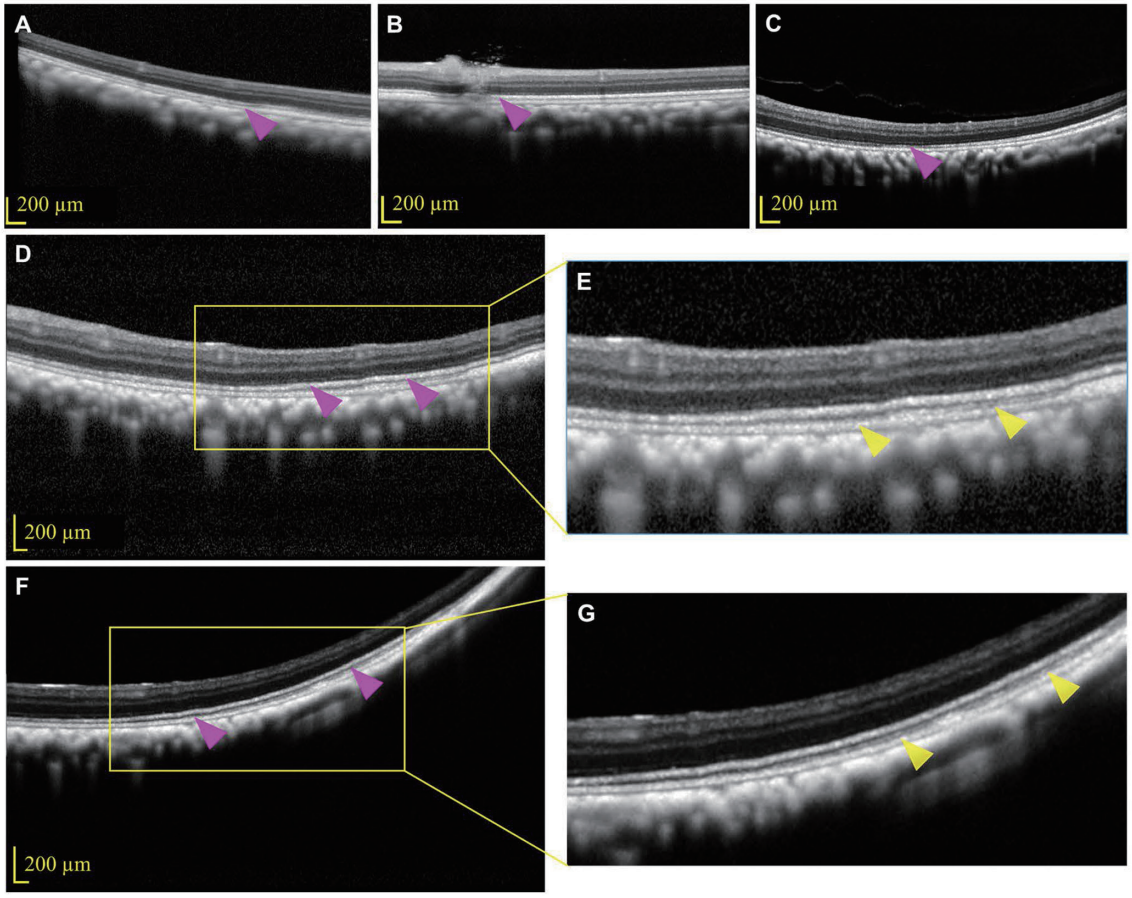

Fig. 5. OCT images of monkey eyes taken on the day after subretinal injection (around 24 hr after injection) with 50 (A), 100 (B), 150 (C), 200 (D and E), and 250 (F and G) µL/eye of physiological saline. Enlarged views of the yellow frames in D and F are shown in E and G, respectively. The blebs nearly disappeared (A-C). Very small interspaces were observed between the photoreceptors and retinal pigment epithelium at the bleb site after injections of 200 and 250 µL (yellow arrow heads in E and G, respectively). Irregularities in the photoreceptor layers were observed at the bleb site after injections of 50 to 250 µL (pink arrow heads).

after injection. Subretinal injection is a method of injection to the region between the retinal photoreceptor layer and RPE, and retinal photoreceptor cells are supplied by choroidal blood vessels through the RPE (Hurley, 2021). We therefore examined the ERG of all monkeys injected subretinally. The ERG a-wave is the 'light' current and basically reflects the reduction in the 'dark' currents due to light absorption in the photoreceptor outer segments and closure of cGMP-gated cationic channels (Penn and Hagins, 1969; Sillman *et al*., 1978; Robson *et al*., 2003; Robson *et al*., 2022). The theoretical origin of the b-wave in ERG is in the bipolar, Müller, and amacrine cells (Stockton and Slaughter, 1989; Tian and Slaughter, 1995). Significant differences from pre-dosing values were found in the latency of the b-wave of monkeys treated with 250 µL/eye and in the amplitude of it with 200 and 250 µL/eye. In the a-wave, while there were no significant differences, the mean percentage after injection relative to pre-injection values were 94.5, 97.6, 86.0, and 77.6% at 100, 150, 200, and 250 µL/eye, respectively. The longer the time that photoreceptor cells and RPE (which are the source of nutrients) remained separated after injection, or the larger the injection volume, the greater the effect on the photoreceptor cells. In particular, in monkeys treated with 200 and 250 µL/eye of physiological saline, residual fluid was observed in the bleb even on the day after injection, suggesting that the choroidal nutrient supply to the photoreceptor cells was affected.

Subretinal injection, in other words, results in an arti-

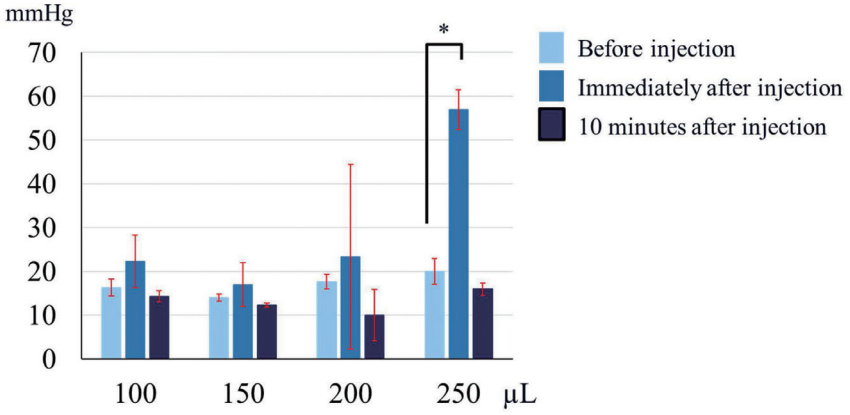

Fig. 6. IOP changes in monkeys given subretinal injections of 100, 150, 200, and 250 µL at a rate of 10 µL/sec. Mean ± standard deviation (n = 3 eyes/group). *P < 0.05 (paired t-test).

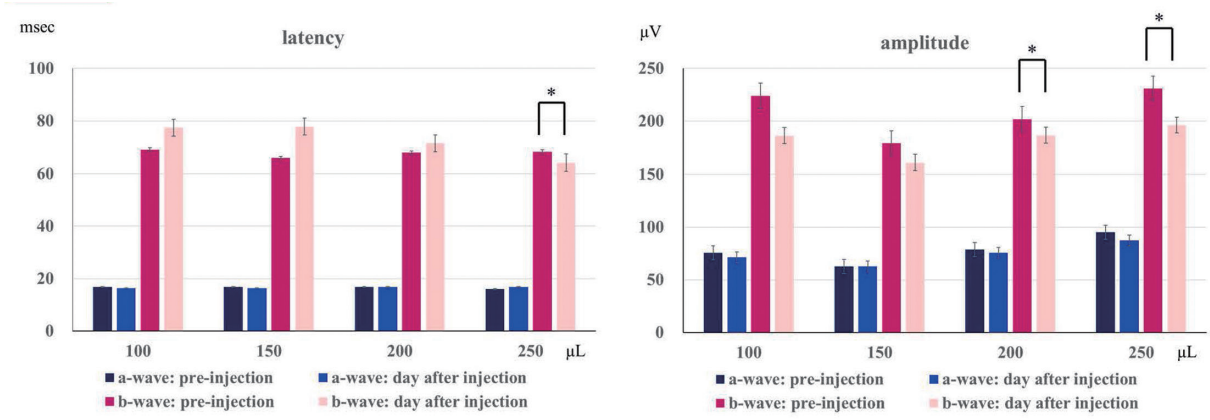

Fig. 7. Latencies and amplitudes of a- and b-waves in the ERG examination in monkeys before (pre-injection) and the day after the subretinal injection of 100, 150, 200, and 250 µL of physiological saline. Mean ± standard deviation (n = 2 eyes/group). *: P < 0.05 (paired t-test).

ficial state of retinal detachment, and retinal detachment is known to cause retinal remodeling (Lewis *et al*., 1998; Mandal *et al*., 2011). Bleb diameter increased with increasing injection volume, indicating that the extent of retinal remodeling could also increase with the injected volume, which could affect synaptic transmission in the retina. Therefore, larger volumes may have resulted in decreases in the latency of the b-wave in eyes injected with 250 µL/eye and the amplitude of the b-wave in eyes injected with 200 and 250 µL/eye.

In conclusion, due to the risk of increased IOP, subretinal injections of 5 µL/eye/min in rats and 150 µL/eye in monkeys were considered favorable as the maximum injection volumes. It should be acknowledged that these results are for saline solution, and in actual use, outcomes may be influenced by the physical properties of the test material. The risks and benefits of subretinal injections at large volumes should be considered in both nonclinical and clinical studies. The data obtained in this study from 2 experimental animal species could contribute significantly to the research of therapies that use subretinal injections.

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Conflict of interest---- The authors declare that there is no conflict of interest.

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