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Movement of retinal vessels toward the optic nerve head after increasing intraocular pressure in monkey eyes with experimental glaucoma

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ABSTRACT

A shift or displacement of the retinal blood vessels (RBVs) with neuroretinal rim thinning indicates the progression of glaucomatous optic neuropathy. In chronic open angle glaucoma, individuals with RBV positional shifts exhibit more rapid visual field loss than those without RBV shifts. The retinal vessels reportedly move onto the optic nerve head (ONH) in response to glaucoma damage, suggesting that RBVs are pulled toward the ONH in response to increased cupping. Whether this phenomenon only applies to RVBs located in the vicinity or inside the ONH or, more generally, to RBVs also located far from the ONH, however, is unclear. The aim of this study was to evaluate the movement of RBVs located relatively far from the ONH edge after increasing intraocular pressure (IOP) in an experimental monkey model of glaucoma.

Fundus photographs were obtained in 17 monkeys. High IOP was induced in the monkeys by laser photocoagulation burns applied uniformly with 360° irradiation around the trabecular meshwork of the left eye. The right eye was left intact and used as a non-treated control. Considering the circadian rhythm of IOP, it was measured in both eyes of each animal at around the same time-points. Then, fundus photographs were obtained. Using Image J image analysis software, an examiner (N.E.) measured the fundus photographs at two time-points, i.e. before laser treatment (time 1) and the last fundus photography after IOP elevation (time 2). The following parameters were measured (in pixels): 1) vertical diameter of the ONH (DD), 2) distance from the ONH edge to the first bifurcation point of the superior branch of the central retinal vein (UV), 3) distance from the ONH edge to the first bifurcation point of the inferior branch of the central retinal vein (LV), 4) ONH area, and 5) surface area of the cup of the ONH. We calculated the ratios of UV to DD (UV/DD), LV to DD (LV/DD), and the cup area to disc area ratio (C/D).

The mean UV/DD at time 1 (0.656 ± 0.233) was decreased at time 2 (0.542 ± 0.192) ($p < 0.01$), and the mean LV/DD at time 1 (0.642 ± 0.151) was decreased at time 2 (0.534 ± 0.171) ($p < 0.01$). The mean C/D at time 1 (0.303 ± 0.035) was increased at time 2 (0.556 ± 0.110) ($p < 0.01$). The mean IOP at time 1 was 19.8 ± 2.5 and that at time 2 was 54.2 ± 15.8. The amount and rate of the change in LV/DD and C/D between time 1 and time 2 were significantly correlated ($r = -0.654$ and -0.536, $p = 0.004$ and 0.026, respectively).

Therefore, in an experimental monkey model of glaucoma, RBVs located relatively far from the ONH were pulled toward the ONH as cupping increased.

1. Introduction

In addition to increased cupping in the optic nerve head (ONH), optic nerve neuroretinal rim thinning, and retinal nerve fiber layer

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thinning, a shift or displacement of the retinal blood vessels (RBVs) with neuroretinal rim thinning indicates the progression of glaucomatous optic neuropathy (Alward et al., 2015; Radcliffe et al., 2014; Sehi and Greenfield, 2006; Varma et al., 1987). Radcliffe et al. (2014) found that individuals with chronic open angle glaucoma exhibiting RBV positional shifts show more rapid visual field loss than those without RBV shifts. Alward et al. (2015) reported that RBVs move onto the ONH in response to glaucoma damage, suggesting that increased cupping of the ONH induced by high pressure (Shimazawa et al., 2012) draws RBVs around the ONH toward the cupping. Whether this phenomenon applies only to RVBs located in the vicinity of or inside the ONH or, more generally, to RBVs located far from the ONH, however, is unclear. If RBVs around the ONH are pulled toward the center of the ONH with increased cupping, retinal nerve fibers may also move toward the cupping, because RBVs are present mainly in the retinal nerve fiber layer.

In the present study, we evaluated the movement of RBVs located relatively far from the ONH edge after increasing IOP in an experimental monkey model of glaucoma.

2. Materials and methods

2.1. Animals

Fundus photographs were obtained in 17 monkeys. Some of the monkeys were used previously for other studies (Shimazawa et al., 2012) (Table 1).

Monkeys No.1–12 were adult male cynomolgus monkeys (*Macaca fascicularis*, 3–4 years old, SNBL DSR, Kagoshima, Japan) selected by stepwise screening from a cohort of 40 animals before the induction of experimental glaucoma. The selected animals exhibited no abnormalities in routine hematology, blood chemistry, or ophthalmology examinations, had lower intra-and inter-day variance in IOP, and were sensitive to IOP-lowering drugs. Animals housed individually in stainless steel cages (68 cm D × 62 cm W × 77 cm H) were maintained at 23–29 °C with relative humidity 30%–70%, ventilation 15 times/h, and artificial lighting for 12 h (7:00 to 19:00), and were provided *ad libitum* access to water and approximately 108 g of solid food (HF Primate J 12G5K9J, Purina Mills, LCC) daily. All procedures performed at SNBL DSR were approved by the Institutional Animal Care and Use Committee of SNBL DSR, which is fully accredited by AAALAC International, and were in accordance with standards published by the National Research Council (Guide for the Care and Use of Laboratory Animals, NIH OACU) of the National Institutes of Health Policy on Human Care and Use of Laboratory Animals.

Monkeys No. 13–17 were adult male cynomolgus monkeys (*Macaca facicularis*, 3–4 years old, Japan SLC Co. Ltd, Hamamatsu, Japan) that were housed in an air-conditioned room at 22–26 °C with 50%–70% humidity, and provided solid food and water *ad libitum*. Animal welfare practices and steps taken to ameliorate suffering were in accordance with the recommendations of the Weatherall report on the use of non-human primates in research, and all investigations that were performed at RIKEN were approved and monitored by the Institutional Animal Care and Use Committee of Kobe Institute in RIKEN.

Table 1
Characteristics, IOP and photo day of 17 cynomolgus monkeys.

No.	Age	Sex	IOP time1 (mmHg)	IOP time2 (mmHg)	Photo day (day)
1	3Y 10M	Male	19	32	62
2	4Y 3M	Male	16	58	62
3	3Y 6M	Male	18	58	62
4	3Y 11M	Male	19	76	62
5	3Y 11M	Male	20	62	62
6	4Y 2M	Male	19	54	62
7	4Y 3M	Male	20	62	62
8	3Y 9M	Male	19	68	62
9	3Y 9M	Male	20	22	62
10	4Y 11M	Male	16	56	62
11	4Y 1M	Male	19	65	62
12	4Y 2M	Male	18	36	62
13	3Y 9M	Male	24	26	63
14	3Y 7M	Male	23	72	27
15	4Y 0M	Male	20	60	78
16	4Y 0M	Male	25	54	106
17	3Y 4M	Male	22	60	165

Y: years old, M: month, IOP: intraocular pressure.

2.2. Induction of experimental glaucoma

In monkeys No.1–12, an increase in the IOP was induced by green laser photocoagulation burns applied at a wavelength of 532 nm for uniform 360° irradiation around the trabecular meshwork of the left eye. The right eye was left intact and used as a non-treated control. The animals were anesthetized with an intramuscular injection (0.2 mL/kg) of a 7:1 mixture of ketamine hydrochloride (50 mg/mL, Supriya Lifescience Ltd.) and xylazine (2% Celactal®, Bayer Yakuhin Ltd.) before laser treatment. An ocular single mirror gonio lens was placed directly on the cornea along with a mucous membrane-protecting agent (Scopizol® solution, Senju Pharmacetical Co.,Ltd.). A 532-nm green laser (spot size; 100 μm, power; 1000 mW, exposure time; 0.2 s) was applied using a Multicolor Scan Laser Photocoagulator (MC-500,NIDEK Co.,Ltd.). The first laser treatment for each animal comprised a total of 60–74 laser beam spots. Additional laser treatment was applied 2 weeks after the first laser treatment.

In monkeys No.13–17, IOP was increased by applying argon-laser burns to the mid portion of the trabecular meshwork of the left eye (Quigley and Hohman, 1983; Shimazawa et al., 2006). An argon blue/green laser was focused on the mid-portion of the trabecular meshwork, and a total of 100–150 laser-beam spots was applied around 360° (spot size, 100 μm; power, 1000 mW; exposure time, 0.2 s) using an argon-laser photocoagulator (Ultima 2000 SEH; Coherent, Inc., CA, USA) attached to a standard slit-lamp microscope (BQ 900; Haag-Streit, Koeniz, Switzerland). Two weeks after the first laser treatment, the treatment was repeated, except for in monkey No.17, to maintain the increased IOP.

2.3. IOP measurements and fundus photography

In monkeys No.1–12, IOP measurements were conducted in the morning twice weekly. At each measurement point, IOP (mmHg) was determined bilaterally with a TonoVet tonometer (TV01, Tiolat Oy) in conscious animals restrained using custom-made restraints. After instilling the mydriatic solution (Mydrin®-P ophthalmic solution, Santen Pharmaceutical, Co., Ltd.), both fundi of each animal were photographed using an ocular fundus camera (Genesis™-Df, Kowa Co., Ltd.) under anesthesia with an intramuscular injection (0.2 mL/kg) of a 7:1 mixture of ketamine hydrochloride (50 mg/mL,) and xylazine (2% Celactal®) before the laser treatment and 62 days after the first laser treatment.

In monkeys No.13–17, IOP was measured in both eyes of each animal using a calibrated pneumatonometer (Model 30 Classic Pneumatonometer; Beaver-Visitec International, Waltham, MA, USA) under ketamine anesthesia (8.75–10 mg/kg, i.m.), with local anesthesia using 0.4% oxibuprocaine hydrochloride (BenoxilH 0.4% solution; Santen Pharmaceutical Co. Ltd.). IOP was measured between 14:00 and 16:00 at 1- or 2-week intervals. Fundus