ASSESSMENT OF THE RETINAL NERVE FIBER LAYER OF THE NORMAL AND GLAUCOMATOUS MONKEY WITH SCANNING LASER POLARIMETRY

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ABSTRACT

Purpose: To describe and test a method for assessment of the monkey retinal nerve fiber layer (RNFL) with scanning laser polarimetry.

Methods: A scanning laser polarimeter was modified to accommodate a variable corneal polarization compensator. Corneal polarization magnitude (CPM) and corneal polarization axis (CPA) of the anterior segment birefringence of normal and glaucomatous cynomolgus monkey eyes were determined from a polarimetry image of the Henle fiber layer. Next, the variable compensator was adjusted to minimize the anterior segment birefringence. RNFL measurements were then obtained. All images were compared with simultaneous optic disc stereoscopic photographs.

Results: CPM was small in each of the eyes examined, ranging from 5.7 nm to 9.0 nm. CPA ranged from -62˚ to 79˚. (Nasally upward CPA values were recorded as negative; nasally downward CPA values were recorded as positive.) When eye-specific compensation was used, RNFL retardation profiles mimicked the expected appearance of the RNFL in all eyes. We also observed a substantial decrease in retardation in eyes with experimental glaucoma compared with healthy fellow eyes.

Conclusions: Individualized anterior segment compensation can be achieved in the monkey eye so that the measured birefringence appears to largely reflect the birefringence of the RNFL. Observed differences in retardation between healthy eyes and eyes with experimental glaucoma suggest that scanning laser polarimetry may be useful for detecting and monitoring RNFL loss in experimental primate glaucoma.

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INTRODUCTION

Scanning laser polarimetry, an optical imaging technique based on the birefringence of the retinal nerve fiber layer (RNFL), has been used to obtain reproducible quantitative and objective measurements of the RNFL in human eyes.[1-5] Light refracted from the RNFL (an anisotropic structure) is polarized, resulting in two refracted rays. One of the rays travels with the same velocity along the optical axis of the tissue (fast axis of polarization), while the other ray travels with a velocity that is dependent on the propagation direction within the tissue (slow axis of polarization). The distance of separation (retardance) between the two rays increases with increasing tissue thickness. However, the RNFL is not the only birefringent structure of the eye. The cornea and, to a much lesser extent, the lens also exhibit birefringence. Further, the Henle fiber layer of the macula is birefringent, consisting of elongated photoreceptor fibers extending radially from the fovea.

Because all birefringent structures cause a change in the polarization of an illuminating beam, the total retardance of a beam illuminating the parapapillary retina consists largely of contributions from the RNFL and cornea.[6] The accuracy of RNFL measurements with scanning laser polarimetry depends on the ability to extract the RNFL retardance from the measured total retardance. To compensate corneal birefringence, the commercial scanning laser polarimeter (SLP) (GDx Nerve Fiber Analyzer, Laser Diagnostic Technologies, Inc, San Diego, California) employs an integrated component that assumes all imaged eyes have a slow corneal polarization axis (CPA) of 15˚ nasally downward with a corneal polarization magnitude (CPM) of 60 nm.

In 1995, both eyes of 11 cynomolgus monkeys were imaged with the commercially available SLP with a fixed corneal compensator (R.N. Weinreb, unpublished data). Each of the monkeys had experimental glaucoma of the right eye and a healthy left eye. Upon inspection of the resulting images, it was apparent that the detected

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retardation (change in polarization due to birefringence) did not mimic the expected appearance of the RNFL in several of the eyes. Figure 1 shows an optic disc photograph (A) and fixed-compensator SLP retardation (thickness) map (B) that were acquired from a monkey with experimental glaucoma of the right eye. Although this monkey had marked narrowing of the right neuroretinal rim (see Figure 1A), the retardation map indicated high retardance. The healthy left eye of the same monkey (Figures 1C and 1D) also had high retardance. The uniformly high retardance observed with this monkey was characteristic of the retardance in each of the other monkeys. On the basis of these observations, it was hypothesized by one of us (R.N. Weinreb) that the CPM and/or CPA in monkey eyes was significantly different from that assumed by the fixed-compensator SLP, and that only by individually compensating for corneal birefringence would it be possible to obtain retardance measurements that reflected the appearance of the monkey RNFL. However, it was not possible to measure CPM and CPA, and this hypothesis could not be tested at that time.

Recently, the commercially available SLP was modified by integrating a variable corneal polarization compensator (VCC) that allows compensation for individual eyes.[7] Using this modified instrument, we have measured CPM and CPA in human eyes.[8] Further, we have corrected SLP images using this information and determined that individually compensated images more closely resemble RNFL appearance than those acquired with a fixed-compensator SLP (unpublished data). The purpose of the current study was first to determine CPM and CPA in individual monkey eyes. Next, we sought to determine whether the VCC SLP provided retardation measurements that resembled what is expected in monkey eyes with and without experimental glaucoma.

METHODS

SUBJECTS

Six eyes of three adult cynomolgus (*Macaca fascicularis*) monkeys were studied. Monkey 1 (age about 7 to 10 years; young adult) had healthy eyes. The left eyes of both monkeys 2 and 3 were healthy. Monkey 2 (aged 16 years) had experimental glaucoma (right eye) of 12 years duration with considerable narrowing of the neuroretinal rim visible on simultaneous stereoscopic optic disc photographs. Monkey 3 (aged 14 years) also had experimental glaucoma (right eye) of 12 years duration, with complete absence of neuroretinal rim visible on optic disc photographs. Experimental glaucoma had been induced in the right eyes of monkeys 2 and 3 by argon laser applications to the trabecular meshwork, according to the methods of Gaasterland and Kupfer.[9]

PROCEDURE

To facilitate SLP imaging, the monkeys were anesthetized with intramuscular ketamine. An intravenous catheter and endotracheal tube were placed. The animals were positioned in a restraint in the prone position with the upper jaw on a bite bar and soft pressure points at the rear of the cranium for head stabilization. Following a bolus intravenous dose of norcuronium (neuromuscular blocker), the animals were connected to a ventilator and vital signs were continuously monitored. Anesthesia was maintained with periodic intramuscular ketamine, and neuromuscular block was maintained with steady infusion of norcuronium.

SCANNING LASER POLARIMETRY

Polarimetry images were obtained using a commercial SLP (GDx, Laser Diagnostic Technologies, Inc, San Diego, California) modified so that the original fixed corneal compensator was replaced with a VCC as described by Zhou and Weinreb.[7] Briefly, the VCC SLP is composed of a set of four linear retarders in the path of the measurement beam. The first two retarders are optical lenses that have equal retardance and form a variable cornea and lens compensator. The third retarder is composed of the cornea and lens, and the fourth retarder is the retinal birefringent structure (RNFL or macular Henle fibers).

The CPM and the CPA were determined by setting the compensating retarders to 0 nm and imaging the macula. The resulting retardation profile represented the additive effects of cornea, lens, and macular Henle fiber birefringence. The compensating retarders were then adjusted to minimize the effects of anterior segment birefringence, resulting in a flat low macular retardation profile. The CPM and CPA values resulting in adequate compensation were then recorded. Three macula images were obtained from both eyes of each monkey to determine mean CPM and CPA. Nasally upward CPA values (degrees) were recorded as negative; nasally downward CPA values were recorded as positive.

Next, three corneal birefringence-compensated parapapillary SLP images from each eye were obtained using the appropriate eye-specific CPM and CPA values. These three images were combined to create each composite image used for RNFL thickness analysis. In these images, each pixel is color-coded to represent the measured retardation, resulting in a retardation map.

Differences in 17 VCC SLP parameters between the right and left eyes of one healthy monkey and the glaucomatous right eyes and healthy left eyes of two other monkeys were examined to determine if SLP with eye-specific corneal birefringence compensation could detect differences between healthy eyes and those with RNFL damage. The investigated parameters were as follows:

superior maximum thickness, inferior maximum thickness, symmetry, superior nasal thickness ratio, superior temporal thickness ratio, inferior temporal thickness ratio, average thickness, ellipse modulation, maximum modulation, total polar integral, superior polar integral, inferior polar integral, ellipse average thickness, superior average thickness, temporal average thickness, inferior average thickness, and nasal average thickness. Each of these parameters has been described in detail previously.[10,11] Although thickness measurements are reported in micrometers (μm), caution should be used when assessing these measurements. This is because the relationship between SLP retardance and RNFL thickness is not known. Therefore, thickness measurements should be considered relative, not absolute.

All experimental procedures adhered to the guidelines of the Association for Research in Vision and Ophthalmology statement for the use of animals in ophthalmic and vision research.

RESULTS

CPM AND CPA MEASUREMENTS IN MONKEY EYES

The measured CPM was small in the six eyes examined, ranging from 5.7 to 9.0 nm. CPA ranged from -62° to 79°. (Nasally upward CPA values were recorded as negative; nasally downward CPA values were recorded as positive.) CPM and CPA of each eye from each subject are shown in Table I.

Monkey 1: Normal Eyes

Figure 2 shows optic disc photographs and VCC SLP retardation (thickness) maps for the right (A and B) and left eyes (C and D) of monkey 1 (both eyes healthy). The retardation maps have areas of increased retardation in the superior and inferior arcuate bundles in accordance with the expected appearance of the thicker RNFL in these regions of healthy eyes. Figure 2E shows the RNFL thickness profile for both eyes, where the x-axis represents the polar position around the optic disc and the y-axis represents RNFL thickness measured in millimeters. The measurements of parapapillary retina thickness are obtained 1.7 optic disc diameters from the optic disc margin. A large-amplitude double-hump pattern, indicative of increased thickness of the superior and inferior arcuate bundles, is apparent, and there is little difference in RNFL thickness between eyes.

Monkeys 2 and 3: Experimental Glaucoma of Right Eye

Figure 3 shows optic disc photographs and VCC SLP retardation maps for the right and left eyes of monkeys 2 and 3.

The optic disc photograph of the right eye of monkey 2 (Figure 3A) indicates considerable narrowing of the neuroretinal rim. The retardation map (Figure 3B) shows reduced retardation, particularly superiorly, in accordance with the thin neuroretinal rim in the optic disc photograph. The optic disc photograph and retardation map from the healthy left eye are shown in Figures 3C and 3D, respectively. Figure 3E shows the RNFL thickness profile for both eyes on the same plot. The RNFL thickness of the eye with experimental glaucoma (OD) shows thinning and a reduced-amplitude double-hump pattern, particularly in the superior quadrant, compared with the healthy left eye.

The optic disc photograph of the right eye of monkey 3 (Figure 3F) indicates complete absence of neuroretinal rim. The retardation map (Figure 3G) indicates uniformly low retardance in accordance with the absence of neuroretinal rim in the optic disc photograph. The optic disc photograph and retardation map from the healthy left eye are shown in Figures 3H and 3I, respectively. Figure 3J shows the RNFL thickness profile for both eyes on the same plot. The retardation from the eye with experimental glaucoma (OD) is markedly reduced superiorly and inferiorly, and the thickness profile does not have a double-hump pattern.

RNFL THICKNESS PARAMETERS

Table II shows RNFL thickness parameter measurements from the right and left eye of each monkey. The values are similar in each eye for monkey 1 (both eyes normal) and are quite different for monkeys 2 and 3 (glaucomatous right eye, normal left eye), with greater differences between eyes for monkey 3. For monkey 2, superior RNFL measurements (superior maximum and superior average), inferior RNFL measurements (inferior maximum and inferior average), and global RNFL measurements (average thickness and ellipse average thickness) in the eye with experimental glaucoma are 75% to 100% of those in the healthy eye. For monkey 3, superior RNFL measurements, inferior measurements, and global measurements in the eye with experimental glaucoma are 36% to 60% of those in the healthy eye.

DISCUSSION

The most notable finding in our study is the fact that the monkey anterior segment birefringence is quite different from that described in humans, as hypothesized. Moreover, scanning laser polarimetry with variable anterior segment polarization compensation appears to objectively and quantitatively measure RNFL thickness in monkey eyes.

Currently, the commercially available SLP assumes a CPM of 60 nm and a CPA of 15° based on measurements from human eyes. Previous studies have shown that in healthy human eyes, CPM varies from 0 to 125 nm, with a mean of approximately 40 nm, a median of approximately

40 nm, and a mode between 40 nm and 50 nm.[8,12] In the current study, CPM in each of the six monkey eyes was in a narrow range, from 5.7 to 9.3 nm. Although the sample is small, these values appear quite different from values in human eyes. In contrast to the narrow range of CPM values, the range of CPA in the monkey was wide and ranged from -62˚ to 79˚. This considerable variability is similar to that reported in humans for CPA values.[8,12,13] The CPA in only one eye (monkey 1, OD, CPA = 27˚) was within 15˚ of the value assumed by the commercially available fixed-compensator GDx (15˚), indicating that SLP RNFL thickness measurements using the commercially available instrument that relies on a fixed corneal birefringence compensation are likely inaccurate in the monkey eye. In

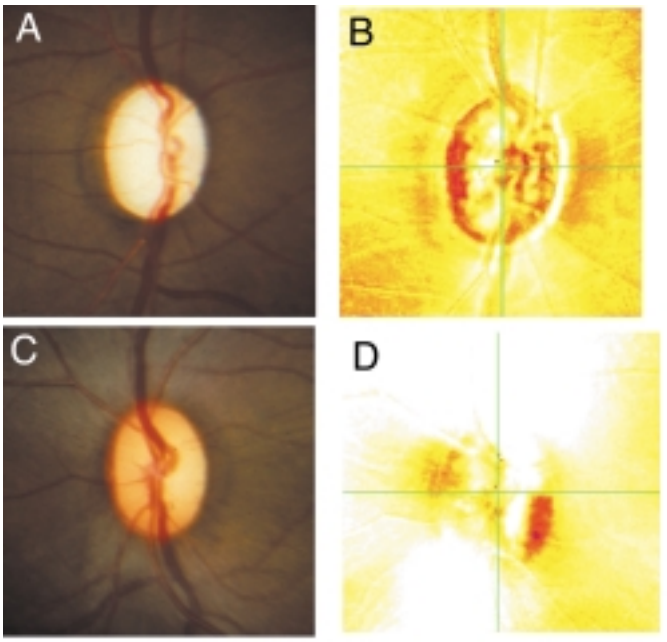

FIGURE 1

Optic disc photographs (A and C) and fixed-compensator scanning laser polarimetry retardation maps (B and D) from monkey with experimental glaucoma of right eye (A and B) and healthy left eye (C and D) (see text for description).

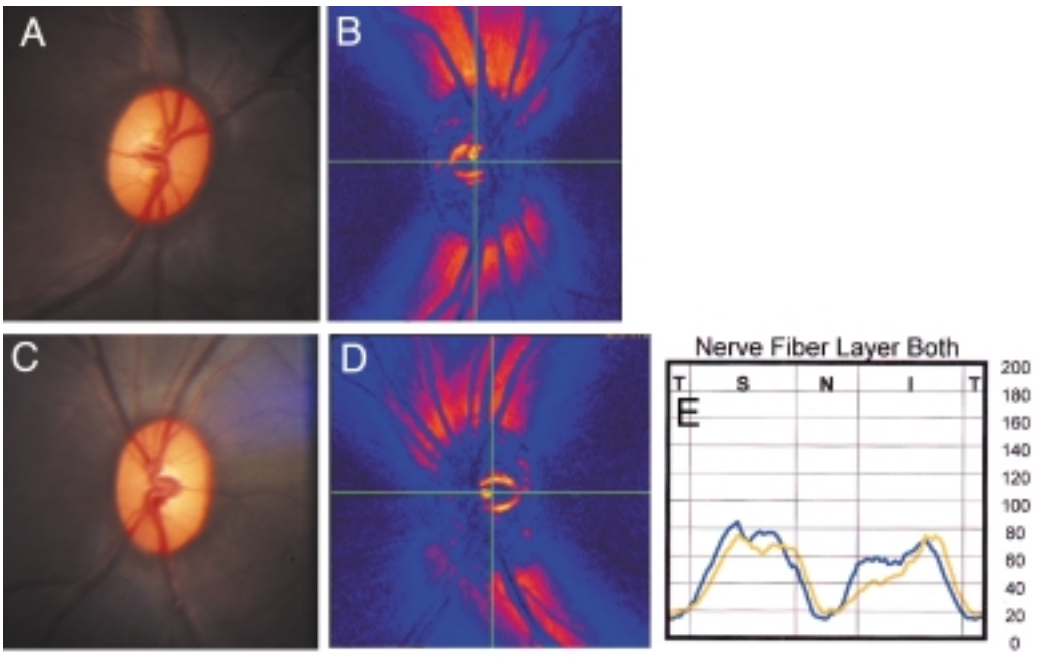

FIGURE 2

Optic disc photographs (A and C) and scanning laser polarimetry (SLP) retardation maps (B and D) using SLP with eye-specific corneal birefringence compensation from a monkey with normal eyes (monkey 1; see text for description). The x-y plot (E) shows parapapillary retinal nerve fiber layer thickness (y-axis) as a function of polar position around the optic disc (x-axis) in both eyes.

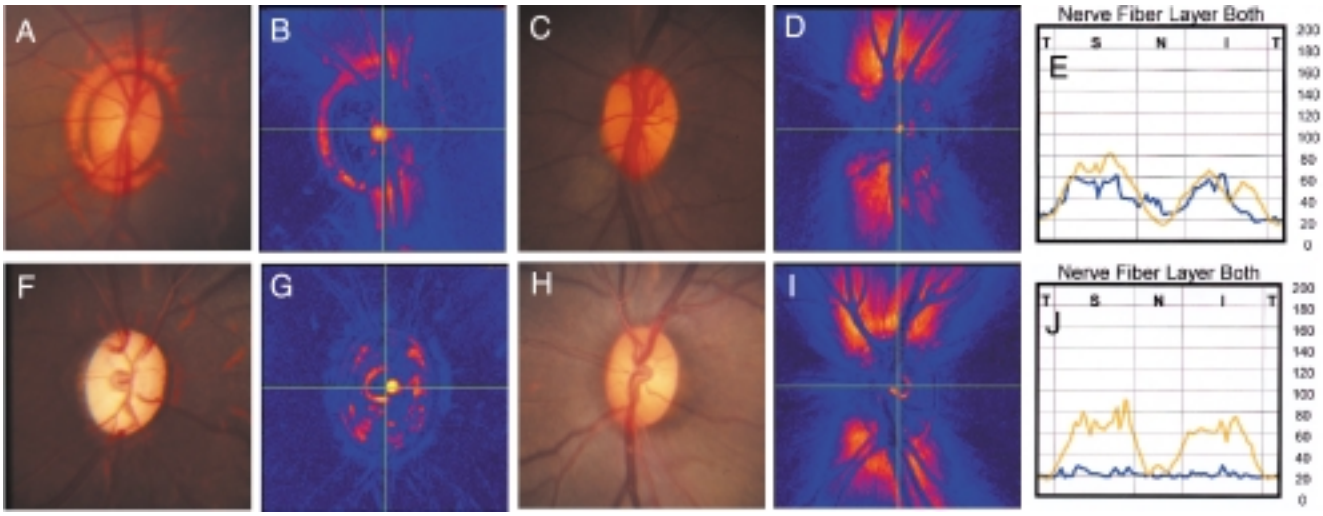

FIGURE 3

Optic disc photographs and scanning laser polarimetry (SLP) retardation maps using SLP with eye-specific corneal birefringence compensation from two monkeys with experimental glaucoma of right eye and normal left eyes (monkey 2, A through D, and monkey 3, F through I; see text for description). The x-y plots (E and J) depicts parapapillary retinal nerve fiber layer thickness (y-axis) as a function of polar position around the optic disc (x-axis) in both eyes of monkey 2 and monkey 3, respectively.

TABLE I: CORNEAL POLARIZATION MAGNITUDE (CPM) AND CORNEAL POLARIZATION AXIS (CPA) OF EACH EYE EVALUATED

SUBJECT	EYE	STATUS	CPM (NM)	CPA
Monkey 1	OD	Healthy	8	27°
	OS	Healthy	8	53°
Monkey 2	OD	Experimental glaucoma	8	-62°
	OS	Healthy	5.7	-44.3°
Monkey 3	OD	Experimental glaucoma	7	78.7°
	OS	Healthy	8	63.7°

retrospect, the SLP images of monkey eyes obtained in 1995 were inaccurate in large part owing to incorrect compensation of corneal birefringence. Testing in additional monkeys is warranted to better validate this suggestion because it is possible that some monkeys have CPM and CPA values that closely approximate those observed in human eyes.

In the current study, there were much larger ocular asymmetries in most RNFL measurements from the monkeys with monocular glaucoma (monkeys 2 and 3) compared to the binocularly normal monkey (monkey 1). This finding indicates that differences in RNFL thickness between healthy and glaucomatous monkey eyes are detectable with SLP, as they are in humans.[4,14-20] Although the sample size is small, we believe that these results are compelling and are applicable to studies using the monkey experimental glaucoma model.

In the monkey model of glaucoma, the optic disc and RNFL change rapidly over several months, in contrast to the chronic and generally slowly progressing course of primary open-angle glaucoma. Results from the current study suggest that SLP with eye-specific corneal birefringence compensation can aid in the evaluation of the rapid change in RNFL thickness measurements from monkey eyes with experimental glaucoma. This may be particularly useful for evaluating the efficacy of new glaucoma therapies that purport to slow or halt the progression of disease. The present results are a first necessary step in addressing the use of SLP as a method of monitoring these effects.

Overall, the current study demonstrates that scanning laser polarimetry of the RNFL is possible in monkey eyes using variable corneal birefringence compensation, and that the resulting measurements reflect observable morphology. In addition, the large difference in SLP parameters between healthy monkey eyes and those with experimental glaucoma has implications for the future use of SLP in studies using the primate model of experimental glaucoma.

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TABLE II: VCC GDX PARAMETER MEASUREMENTS FROM BOTH EYES OF EACH MONKEY*

	MONKEY 1 (HEALTHY OU)		MONKEY 2 (GLAUCOMA OD, HEALTHY OS)		MONKEY 3 (GLAUCOMA OD, HEALTHY OS)	
	OD	OS	OD	OS	OD	OS
Superior maximum thickness	69.6	60.0	46.7	57.7	24.8	64.1
Inferior maximum thickness	56.0	59.8	38.9	52.0	22.7	57.4
Symmetry	1.24	1.00	1.20	1.11	1.09	1.12
Superior nasal ratio	4.51	3.61	1.85	3.47	1.36	3.73
Superior temporal ratio	5.16	4.06	2.72	4.00	1.34	4.56
Inferior temporal ratio	4.15	4.06	2.25	3.61	1.23	4.08
Average thickness	37.0	36.0	38.0	37.0	22.0	39.0
Ellipse modulation	5.05	3.57	2.52	4.25	0.65	5.00
Maximum modulation	4.16	3.06	1.71	3.0	0.36	3.56
Total polar integral	0.42	0.40	0.36	0.41	0.22	0.43
Superior polar integral	0.19	0.18	0.16	0.19	0.08	0.19
Inferior polar integral	0.16	0.16	0.12	0.16	0.09	0.17
Ellipse average thickness	45.9	44.1	38.8	43.9	20.6	47.1
Superior average thickness	61.3	56.1	47.3	58.5	21.8	60.0
Temporal average thickness	15.7	18.4	24.6	19.8	19.0	17.0
Inferior average thickness	60.0	48.0	37.8	45.9	20.4	53.3
Nasal average thickness	23.2	26.7	32.9	23.0	19.3	27.7

*Thickness measures are expressed in µm.

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DISCUSSION

DR ROBERT L. STAMPER. Over the last half century, the diagnosis and assessment of progression in glaucoma have depended on two basically subjective examinations: visual field testing, which is subjective for the patient, and optic nerve evaluation, which is subjective for the examiner.

The ultimate dream of glaucoma diagnosis and management has been an objective assessment of either visual field or optic nerve that is both sensitive to detect the earliest glaucomatous pathology and/or progression and specific enough to rule out normal variation or artifact. The scanning laser polarimeter (SLP) was touted as such an instrument and, along with two other laser scanning devices based on different principles, has become an increasingly common part of the evaluation of the glaucoma patient. The SLP is based on the true assumption that as glaucoma progresses and the ganglion cells die, that the retinal nerve fiber layer (RNFL) decreases in thickness, and that since the nerve fiber layer is made up of polarizing elements, the polarimeter can detect both relative and absolute thinning of the nerve fiber layer.

The fly in the ointment is that both the cornea and, to a smaller extent, the lens contribute to the polarizing effects of the eye, and these elements can confound the polarized signal coming from the retina. The original SLP was based on the assumption that all human corneas have approximately the same polarizing effect and that the effect was roughly in the same axis in each patient.

It became clear through clinical observations as well as unpublished data in monkeys by the author that the results did not always coincide with the observed appearance of the RNFL. The author and one of his colleagues hypothesized that corneal birefringence was variable from individual to individual and from species to species and that this variability was confounding the results. They developed an individualized, variable corneal birefringence compensator based on macular birefringence and modified the original instrument to incorporate this advance. This study reports their results in three monkeys.

Six eyes from three adult Cynomolgus monkeys were studied while the animals were under general anesthesia. Two eyes of two monkeys had induced glaucoma. The eye specific magnitude and axis of anterior segment (corneal and lenticular) birefringence as well as RNFL parameters were studied.

While the range of corneal polarization magnitude was small (5.7 to 0.0 nm), the corneal polarization axis ranged over 140° from -62° to 79° quite different from humans (range, 0-125; median, 40nm) in magnitude, although similar in axis. The areas of thinning of the nerve fiber layer by SLP matched the observed areas by ophthalmoscopy.

SIGNIFICANCE

This new modification corrects the false assumption of the original polarimeter and appears to give a more accurate assessment of RNFL by eliminating a confounding variable. Thus, assessment of the RNFL thickness both in humans and in monkeys now provides more accurate diagnostic information. The modification also allows better and more objective assessment of the natural history and rate of progression of experimental glaucoma in monkeys as well as clinical glaucoma in humans.

QUESTIONS

I recognize the expenses and difficulties in expanding such a study. However, given the large range of observed polarization axis differences in monkeys even between the two eyes of the same monkey, are three animals, six eyes, enough to be comfortable that the results are representative? Furthermore, the authors have made a good start in validating this device in that the RNFL thickness measurements reflect subjective assessments. However, actual objective assessment of RNFL thickness compared to the SLP measurements would be the ultimate validation.

Corneal polarization magnitude and axis would seem to be less important for longitudinal assessment as they are probably stable over time. However, could these values change with age or other environmental stresses (not to mention LASIK, cataract, etc)? Now that a way of measuring the anterior segment polarization is available, this assumption should be assessed.

I extend my congratulations to the authors for developing and beginning the scientific assessment of a device that should move us considerably closer to the goal of objective measurement of anatomical changes in glaucoma.

DR ROBERT N. WEINREB. The small magnitude of corneal compensation that we have observed has been remarkably uniform in the monkeys that we have examined to date. Testing in larger numbers of monkeys is needed to confirm this observation. Moreover, I agree fully that it is essential to assess longitudinally this technology and to establish the stability of the compensation over time.