Effects of Photorefractive Keratectomy-Induced Defocus on Emmetropization of Infant Rhesus Monkeys

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PURPOSE. To investigate whether photorefractive keratectomy (PRK) performed in infant primates can modify emmetropization and therefore could be used to study mechanisms of refractive error development.

METHODS. Six healthy rhesus monkeys ranging in age from 2 to 3 months were randomly divided into two groups (n = 3 each). Anisometropia was induced in each animal by performing PRK on one eye. Hyperopic anisometropia was induced in group A monkeys by flattening the cornea of the right eye, whereas myopic anisometropia was produced in group B monkeys by steepening the cornea of the right eye. Corneal morphology and topography, refractive status, and axial growth were evaluated over a 5-month observation period.

RESULTS. All the PRK-treated corneas were re-epithelialized and transparent within 3 days after surgery. Subsequently, all the surgically treated eyes exhibited interocular alterations in axial growth rate that were appropriate to compensate for the PRK-induced anisometropia. Specifically, vitreous chamber elongation rates were faster in the eyes with induced hyperopias than in their fellow eyes (0.65 ± 0.05 mm vs. 0.40 ± 0.09 mm), but slower in the eyes with induced myopia than in their fellow eyes (0.58 ± 0.15 mm vs. 0.73 ± 0.10 mm). In some animals, the recovery from the induced anisometropia was facilitated by interocular differences in the rate of corneal flattening. However, the rates of corneal flattening in the treated eyes and their fellow eyes were not significantly different.

CONCLUSIONS. PRK-induced defocus predictably alters axial growth rate and the normal course of emmetropization in developing eyes. Thus, PRK is a useful alternative to current methods used to impose experimental refractive errors in laboratory animals. These results also indicate that refractive surgery performed in childhood may affect normal growth of the eye, resulting in decreased predictability of future refractive status. (Invest Ophthalmol Vis Sci. 2004;45:3806–3811) DOI: 10.1167/iovs.03-0326

Emmetropization is an active and visually guided process in developing eyes of chickens,1–2 tree shrews,2 guinea pigs (McFadden S, et al. IOVS 1995;36:ARVO Abstract 758), marmosets,4 and macaque monkeys,5–7 as evidenced by the fact that optically induced defocus predictably alters the normal course of emmetropization. The most common strategy used to produce defocus involves securing spectacle lenses in front of one or both eyes of an experimental animal. However, spectacle-rearing procedures limit the visual field, can alter the size of the retinal image, and can induce prismatic effects that influence interocular alignment and eye movements. These side effects could confound the aims of the rearing strategies and produce misleading results. In this respect, contact-lens-rearing regimens appear more appropriate than spectacle-rearing regimens for investigation of vision-related emmetropization.8 However, contact-lens-rearing regimens have their own disadvantages. For example, for unknown reasons, extended-wear soft contact lenses produce hyperopic shifts in refractive error in infant rhesus monkeys, regardless of the refractive power of the contact lens.9 This side effect can be avoided by removing the lenses for extended periods each day.10 However, this strategy potentially pits the effects of imposed defocus against the effects of contact lens wear and thus introduces unknown factors into the experiments.

Photorefractive keratectomy (PRK) offers a way to impose refractive errors while avoiding the potentially confounding effects of lens-rearing strategies. In addition, PRK procedures could greatly simplify the care regimen necessary with other methods. For example, frequent cleaning and examination to verify the position of spectacle and contact lenses are avoided with PRK procedures. Another advantage is that PRK procedures can be applied at any age, whereas traditional optical-rearing strategies are feasible only in infants. PRK procedures have been used with some success to study emmetropization in rabbits.10 The purpose of this study was to determine whether PRK-induced alterations in refractive error produces predictable changes in refractive development in infant monkeys.

MATERIALS AND METHODS

Animals

Six healthy rhesus monkeys (Macaca mulatta) ranging in age from 2 to 3 months were used in the study. The use of the animals was approved by The Sun Yat-sen University Institutional Animal Care and Use Committee and was in compliance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. All animals were reared in our animal laboratory under a 12-hour light–dark lighting cycle.

Photorefractive Keratectomy

The six experimental monkeys were randomly assigned to one of two treatment groups (n = 3 each). The monkeys were anesthetized with ketamine hydrochloride (15 mg/kg body weight) and acepromazine maleate (0.2 mg/kg body weight). PRK was performed on one eye of each animal with an excimer laser that was equipped with flying spot and eye-tracking technologies (Technolas 217; Bausch & Lomb, Tampa, FL). The laser-irradiation zone was 5 mm in diameter and centered on the eye’s pupillary axis. The corneal epithelium was intact at the onset of the procedures, and the eyes were stabilized with forceps. The ablations were performed using the same laser parameters that are
normally used in adult humans to correct 3.0 D of myopia (group A) or hyperopia (group B). Thus, the PRK procedures produced relative hyperopic and myopic refractive-error shifts in the treated eyes of the group A and B animals, respectively. For 10 days after surgery, 0.3% tobramycin and 0.1% dexamethasone were topically applied six times each day during the daily light cycle, and 0.3% tobramycin and 0.1% dexamethasone in ointment form were instilled each night.

**Optical and Biometric Measurements**

The eyes were examined weekly for the first 3 weeks after surgery and at monthly intervals for the remainder of the 5-month observation period. All examiners were masked to the treatment group assignment of each animal. The effects of the PRK treatment regimen were assessed by corneal topography, cycloplegic retinoscopy, A-scan ultrasonography, slit lamp biomicroscopy, and applanation tonometry. To make these measurements, the animals were anesthetized with intramuscular injections of ketamine hydrochloride (15 mg/kg body weight) and acepromazine maleate (0.2 mg/kg body weight), and their eyelids were held open with lid retractors. Cycloplegia was induced with 3 drops of topically instilled 0.5% tropicamide 10 minutes apart, 45 minutes before performing the retinoscopy. Retinoscopy was performed by three examiners, and the results were expressed as mean spherical equivalent, spectacle plane refractive corrections. During these measurements, pupil size and the retinoscopy reflex were stable.

**Figure 1.** Photographs of PRK-treated eye (A) and fellow eye (B) in one animal 3 months after PRK.

**Figure 2.** Changes in spherical equivalent refractive error, vitreous chamber depth, and corneal power plotted as a function of time in the group A monkeys with PRK-induced hyperopic anisometropia. Filled symbols: treated eyes; open symbols: fellow eyes. The number designating each monkey is shown within each panel. The first data point is the pre-PRK measurement, and the second data point is the 2-week post-PRK measurement. The panels in the bottom row show interocular differences in refractive error and vitreous chamber depth with time PRK-induced hyperopia. All three animals showed a consistent reduction in the magnitude of the anisometropia and a relative increase in vitreous chamber depth in the PRK-treated eyes.
There was no evidence of residual accommodation. Corneal refractive power was measured with a handheld corneal topographer (Vista; EyeSys, Houston, TX). The depth of the vitreous chamber was measured with A-scan ultrasonography at a velocity of 1532 m/s. The reported values represent the mean of 10 individual measurements. To be acceptable, the standard error for the 10 measurements had to be less than 8 (AXIS-II; Quantel Medical Inc., Clermont-Ferrand, France). Intraocular pressure was measured by a noncontact tonometer (XPERT NCT Plus Advanced Logic Tonometer; Reichert Ophthalmic Instruments, Depew, NY). The reported values represent the means of three individual measures.

Statistics
All measurements were analyzed on computer (SPSS 10.0; SPSS Science, Chicago, IL). Paired t-tests were used to compare the results in the treated and nontreated eyes of experimental monkeys, with statistical significance set at \( P < 0.05 \).

RESULTS

Slit Lamp Biomicroscopy
Re-epithelialization occurred within 3 days after surgery. All surgically treated corneas maintained transparency throughout the observation period, with no signs of any adverse responses from the PRK procedures (Fig. 1).

Intraocular Pressure
Throughout the observation period, the IOPs in all eyes varied from 11 to 14 mm Hg. There were no significant interocular differences in IOP between the surgically treated and fellow nontreated eyes at any time during the measurement period (paired t-test, \( P > 0.05 \)).

Refractive Status
Before the PRK procedures, all the monkeys exhibited the moderate hyperopic errors that are typical of infant rhesus monkeys; the refractive errors ranged from \(+1.25\) to \(+1.71\) D (mean = \(1.42 \pm 0.15\) D). The refractive errors in the left and right eyes were also very similar (paired t-test, \( P = 0.89 \)).

As illustrated in Figures 2 and 3, the PRK procedures produced the desired anisometropia in all six monkeys. Two weeks after surgery, the treated eyes of the group A monkeys were on average \(+2.3\) D more hyperopic than their fellow eyes. In contrast, the treated eyes of the group B monkeys were on average \(-3.9\) D more myopic than their fellow eyes. The initial imposed degree of anisometropia in all the treated ani-
mals fell outside the range of anisometropias observed in normal, untreated monkeys. Over the next 4.5 months, the degree of anisometropia decreased in a systematic manner in all six treated monkeys, with three of the six animals becoming essentially isometric (<0.5 D of anisometropia) by the end of the observation period (Table 1). In all cases, the decrease in anisometropia occurred because the refractive errors of the treated eyes appeared to regress toward those in the non-treated eyes. As seen in the lowest two plots of Figures 2 and 3, which show the interocular differences in refractive error plotted as a function of age, the pattern and time course for the reduction in anisometropia were very consistent within each experimental group. During the postsurgical observation period, the absolute degree of anisometropia decreased in all six treated monkeys. The average changes in the absolute degree of anisometropia in all six treated monkeys decreased significantly from 3.14 D at 2 weeks after PRK to 0.72 D at 5 months after PRK ($t = 5.47; P = 0.006$).

**Vitreous Chamber Depth**

The PRK procedures were performed when the animals were in the very rapid phase of infantile ocular growth; and thus, as expected, all eyes exhibited an absolute increase in vitreous chamber depth during the observation period. However, the key point is that all six treated monkeys exhibited interocular differences in the rate of vitreous chamber elongation. In group A, the more hyperopic treated eyes grew at a faster rate, whereas, in group B, the treated eyes with the imposed myopic errors grew at a slower rate than their fellow nontreated eyes. The interocular alterations in vitreous chamber growth rate were most obvious during the first 3 months after surgery (Table 2). During this period, the vitreous chambers of the group A treated eyes increased 0.23 mm more than those of their fellow nontreated eyes (0.63 ± 0.05 mm vs. 0.40 ± 0.09 mm), whereas the vitreous chambers of the group-B-treated eyes increased 0.15 mm less than those of their fellow nontreated eyes (0.58 ± 0.13 mm vs. 0.73 ± 0.10 mm). For all the treated animals combined, the absolute interocular difference in vitreous chamber depth increased from an average of 0.04 to 0.23 mm at 3 months after PRK ($t = −7.89; P = 0.0002$), and most important, in each case the direction of the vitreous chamber changes were appropriate to eliminate the imposed anisometropia.

**Corneal Topography**

In the group A monkeys, the PRK procedures produced an average relative reduction in corneal power of 1.83 D, which was slightly less than the degree of measured anisometropia. We believe that the mismatch between the measured degree of anisometropia and the initial interocular differences in corneal power reflects the fact that the simulated $K$ values from the topography came from a relatively small part of the cornea, whereas our retinoscopy values were integrated across the entire pupil. Regardless, these induced interocular differences in corneal power were relatively stable throughout the observation period in both group A and B monkeys (Fig. 2; Table 3). Specifically, during the first 3 months after surgery, the rates of reduction in corneal power in the treated and nontreated eyes were identical in group A monkeys (0.008 ± 0.003 D/d treated vs. 0.008 ± 0.001 D/d untreated) and were not significantly different in group B monkeys (0.027 ± 0.006 D/d treated vs. 0.013 ± 0.007 D/d untreated). During the postsurgical observation period, the changes in corneal power for the treated and nontreated eyes were not significantly different ($n = 6, 1.73 ± 1.16 D vs. 1.35 ± 0.45 D$; paired $t$-test, $P = 0.39$). However, as shown in Figure 3, the interocular corneal power differences in the group B monkeys were not as stable over time as the differences in the group A monkeys. Regressions in the treat-

### Table 1. Refractive Status at Different Time Points

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Prior to PRK</th>
<th>2 Weeks PO</th>
<th>3 Months PO</th>
<th>5 Months PO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>OS</td>
<td>PRK</td>
<td>Fellow</td>
</tr>
<tr>
<td>Group A (hyperopic defocus)</td>
<td>1</td>
<td>+1.25</td>
<td>+1.57</td>
<td>+3.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+1.25</td>
<td>+1.30</td>
<td>+3.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+1.50</td>
<td>+1.63</td>
<td>+3.75</td>
</tr>
<tr>
<td>Group B (myopic defocus)</td>
<td>1</td>
<td>+1.25</td>
<td>+1.50</td>
<td>−3.25</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>+1.25</td>
<td>+1.50</td>
<td>−3.25</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>+1.63</td>
<td>+1.50</td>
<td>−2.75</td>
</tr>
</tbody>
</table>

PO, Postoperatively; PRK, eye treated by PRK; Fellow, Fellow eye of the same animal. Data are expressed in diopters.

### Table 2. Vitreous Chamber Depth at Different Time Points

<table>
<thead>
<tr>
<th>Monkey</th>
<th>Prior to PRK</th>
<th>2 Weeks PO</th>
<th>3 Months PO</th>
<th>5 Months PO</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>OD</td>
<td>OS</td>
<td>PRK</td>
<td>Fellow</td>
</tr>
<tr>
<td>Group A (hyperopic defocus)</td>
<td>1</td>
<td>9.84</td>
<td>9.83</td>
<td>9.96</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>9.97</td>
<td>10.01</td>
<td>10.05</td>
</tr>
<tr>
<td>Group B (myopic defocus)</td>
<td>1</td>
<td>9.91</td>
<td>10.01</td>
<td>10.13</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>9.91</td>
<td>9.96</td>
<td>10.11</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>10.02</td>
<td>10.05</td>
<td>10.22</td>
</tr>
</tbody>
</table>

Data are expressed in millimeters. See Table 1 for description of column headings.
ment effect can be seen clearly in animals 1 and 3 in group B. Consequently, some of the reduction in anisometropia observed in these monkeys was due to regression of the PRK treatment effects. It is important to note, however, that the alterations in vitreous chamber growth appear to compensate for the regression in corneal treatment effects. For example, animal 1 of group B initially exhibited an interocular difference in vitreous chamber depth that was in the appropriate direction to compensate for the induced anisometropic error; but, as the degree of optically imposed error regressed, the interocular differences in vitreous chamber depth regressed.

**DISCUSSION**

This study showed, for the first time, that PRK-induced defocus predictably alters vitreous chamber growth rate and the course of refractive-error development in infant primates. Specifically, PRK-induced hyperopic anisometropia promoted a relative acceleration in ocular axial growth in the treated eyes and a myopic shift in the refractive error of the treated eye. In contrast, PRK-induced myopic anisometropia decreased axial growth rates and caused a hyperopic shift in the treated eye’s refractive state. This pattern of results is similar to that observed in infant monkeys reared with anisometropic errors induced with spectacle lenses. In the present study, the vitreous chamber elongation rate appeared to return to normal, with a gradual recovery of normal emmetropization after 3 months of optical defocus, similar to what happened in the eyes with spectacle-lens–induced defocus in monkeys, indicating that the mechanisms that are responsible for the observed alterations in eyes treated with PRK are similar to those that produce compensating changes in animals treated with spectacle lenses. The similarity reinforces the conclusion that the changes in refractive development in spectacle- and PRK-treated animals were regulated by signals related to the changes in effective focus produced by the imposed anisometropias, and not to any possible alterations associated with changes in visual field or interocular differences in retinal image size.

As an experimental treatment strategy, PRK is not without potential problems. In particular, any changes in corneal clarity or integrity could have confounding consequences. This is a significant concern, because the potential reduction in retinal image contrast associated with PRK-induced corneal haze could promote the development of form-deprived myopia. Although alterations in corneal transparency have been associated with wound-healing after PRK procedures in humans, subepithelial haze was not observed in any of the treated eyes at any time during our experiments. Likewise, although increases in IOP have been observed after PRK, we did not find any significant changes in IOP in any of the PRK-treated eyes. Therefore PRK-induced defocus appears to be a suitable technique for studying vision-dependent growth in infant monkeys. One disadvantage of PRK procedures, however, is that unlike traditional spectacle-lens–induced defocus, PRK changes are not readily reversible.

Laser refractive surgery has been used widely for correction of refractive errors for nearly 20 years. However, this procedure is not recommended for young children and adolescents with refractive errors, because their eyes have not completely developed. This practice is based on the assumption that PRK-induced corrections could alter the normal course of emmetropization and that refractive errors would not be stable after laser refractive surgery in early life. The results of this study are in agreement with the idea that PRK-induced changes in refraction that (unlike traditional spectacle lens corrections) are very constant over time and could influence ocular growth.

In summary, this study demonstrated that visual defocus initiated by PRK during infancy in higher primates can influence the emmetropization process, apparently by a mechanism that regulates axial growth in such a way as to eliminate the imposed defocus.

**Acknowledgments**

The authors thank Earl L. Smith III and Li-Fang Hung (College of Optometry, University of Houston, Houston, TX) for good suggestions regarding the experiments; Wang Zheng and Yang Bin for performing the PRK procedures; Ruo Zhong Xie (Vision CRC, University of New South Wales Sydney, Australia) for critical review and scientific suggestions; and Earl L. Smith III and William K. Stell for suggestions for revising the manuscript.

**References**