The Effect of Pirenzepine on Positive- and Negative-Lens–Induced Refractive Error and Ocular Growth in Chicks

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PURPOSE. The selective muscarinic antagonist pirenzepine inhibits experimentally induced myopia in avian and mammalian species, including nonhuman primates and adolescent humans. Transient positive lens defocus has a potent inhibitory effect on negative-lens–induced myopia in avian and mammalian models. The purpose of the present study was to determine the influence of daily treatment with pirenzepine on ocular growth and refractive error in chicks wearing positive lenses.

METHODS. The chicks were allocated to one of eight groups (n = 6 each group) on the basis of whether they wore +10 or −10 D lenses monocularly and whether they received daily intravitreal injections of pirenzepine (700 μg) or vehicle (phosphate-buffered saline) in the lens-defocused eye. In vivo refractive and biometric data were collected, and glycosaminoglycan synthesis in the sclera was assessed.

RESULTS. Pirenzepine did not alter the level of positive-lens–induced hyperopia in chicks wearing +10 D lenses compared with that in the vehicle control group (+8.1 ± 0.6 D vs. +8.9 ± 2.4 D; mean ± SEM; P = 0.76). In contrast, pirenzepine caused significant inhibition of negative-lens–induced myopia compared with that in the vehicle group (−1.1 ± 1.5 D vs. −8.8 ± 1.1 D; P = 0.001). Glycosaminoglycan synthesis in the posterior sclera was significantly increased in the negative-lens–treated groups and showed small decreases in the positive-lens–treated groups.

CONCLUSIONS. The influence of pirenzepine on ocular growth in chicks differed by sign of lens defocus, with pirenzepine blocking negative-lens effects on ocular growth, but not positive-lens effects. The most likely reason that hyperopia was not enhanced by pirenzepine treatment was that the rapid compensatory eye growth associated with positive lenses eliminated the imposed myopic defocus, and the clear retinal image prevented any additional hyperopia from developing. (Invest Ophthalmol Vis Sci. 2010;51:5438–5444) DOI:10.1167/iovs.09-4431

Muscarinic antagonists are a class of drugs that have been examined extensively because of their inhibitory effect on the progression of myopia in humans1–5 and in animal models.6–7 The nonselective broad-band muscarinic antagonists atropine6 and oxyphenonium8 and the selective muscarinic antagonists pirenzepine7–9 and himbacine10 have been shown to be effective in preventing the axial elongation and myopia induced by form deprivation in animal models.

Pirenzepine has been shown to inhibit not only form-deprivation myopia in chicks, tree shrews, monkeys, and guinea pigs,8,11–13 but also negative-lens–induced myopia in tree shrews and guinea pigs.11,13 However, the effects of muscarinic antagonists on positive-lens–induced hyperopia have not been explored in detail, except by Rickers and Schaeffel.7 This group reported that pirenzepine did not stop positive-lens–induced hyperopia in chicks, even at toxic doses that were effective in reducing form-deprivation myopia, while negative-lens–induced myopia was suppressed partially, at the same high doses. However, in the Rickers and Schaeffel study, pirenzepine was administered only once at the start of the experimental period in the lens-defocus paradigm, with an intravitreal injection on day 17 and lens defocus for 4 days. The partial inhibition of both form-deprivation myopia and negative-lens–induced myopia was attributed to retinal toxic mechanisms, rather than physiological receptor-mediated mechanisms.9 As pirenzepine has an elimination half-life of 4 and 7 hours from the retina and sclera, respectively, when administered intravitreally,15 one must assume that any effect of pirenzepine administration in that study was due to the effects of the single dose in the first 12 hours after administration. Thus, it is not surprising that only very high (toxic) doses resulted in any change in ocular growth.

The findings of Rickers and Schaeffel, when using the lens defocus paradigm, were at odds with the results in later studies in which daily intravitreal injections of significantly lower doses of pirenzepine were administered in chicks,8,16 which fully prevented experimentally induced myopia with no evidence of toxic damage to the retina. Pirenzepine prevented the excessive axial elongation accompanying form-deprivation myopia at substantially lower, nontoxic doses in chicks8,16 and tree shrews11 and inhibited negative-lens–induced myopia in tree shrews.11 It has also been found that daily injections of atropine or pirenzepine induce a small degree of hyperopia in unoccluded eyes.5,8

In the present study, we investigated the effects of daily pirenzepine treatment on positive-lens–induced hyperopia. From previous studies, we know that positive lens defocus causes more rapid changes in eye growth over a shorter treatment duration than do equal-powered negative lenses.17–19 Positive-lens–induced hyperopia is more resistant to interruption with periods of normal vision than is negative-lens–induced myopia,20 and durations of positive- and negative-lens defocus do not add linearly and are weighted differently, resulting in induced hyperopia despite only brief episodes of positive lens wear interspersed between day-long periods of negative lens wear.17,18 The marked differences in the temporal integration of the lens defocus signal and its effects on ocular growth between positive and negative lens defocus...
warrant further investigation. In particular, the purpose of the present study was to elucidate further the underlying mechanisms of positive-lens defocus by assessing the influence of pharmacologic intervention with pirenzepine on the development of hyperopia in positive-lens–treated eyes.

Specifically the purpose was to assess whether pirenzepine would work additively with positive-lens treatment to induce hyperopia, in excess of the power of the positive lenses defocus. Both treatments have been shown to inhibit myopic eye growth and also to induce hyperopia when applied to normal open eyes. The combined treatments may provide an enhanced signal to slow ocular elongation and induce hyperopia more than when they are used individually. If they are found to work through separate ocular growth pathways that are additive in effect, it may provide a new treatment approach for the prevention of progression of myopia in humans.

**MATERIALS AND METHODS**

**Animals**

Newly hatched chicks (Gallus gallus domesticus) of the White Leghorn and Black Australorp cross variety were obtained from a local supplier and kept in a temperature-controlled brooder maintained at 28°C, on a 12 hour light–dark cycle. The illumination at the floor of the brooder was 300 lux. The chicks were acclimatized to the conditions of the brooder for 7 days before experimental procedures. Food and water were available ad libitum. All procedures were performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research.

**Experimental Groups and In Vivo Procedures**

The chicks were allocated to one of eight groups (n = 6 each group) on the basis of whether they wore a lens and whether they received an intravitreal injection (7 μL) of pirenzepine (pz) or the vehicle PBS (sal) in the treated eye. The groups were +10 D-pz, +10 D-sal, +10 D-pz, −10 D-sal, +10 D no-injection, −10 D no-injection, open-pz, and open-sal. The +10 D-pz and −10 D-pz were the two main treatment groups, which were given a daily intravitreal injection of 700 μg pirenzepine in the lens-wearing eye. The +10 D-sal and −10 D-sal groups received an intravitreal injection of PBS alone in the lens-wearing eye and acted as the control for the effects of the injection on ocular growth. Two groups of animals were injected intravitreally with pirenzepine (open-pz) or PBS (open-sal) in one eye, and neither eye wore lenses (binocularly open groups). These groups served as the control for the effect of the injected substance on normal ocular growth. Two groups wore +10 or −10 D lenses over one eye and acted as controls for the effect of lenses alone (+10 D no-injection and −10 D no-injection). The lens and/or injection treatments were monocular, and the left eye was the treated eye in all cases. The contralateral eye was left untreated for comparison. Treatment was either started on the seventh day after hatching or was staggered by a day to enable appropriate timing of measurements on the final experimental day. Intravitreal injections were performed as described elsewhere.

The chicks were anesthetized with 2% halothane: the upper lid retracted; the superior temporal anterior sclera viewed under an operating microscope (Carl Zeiss Meditec, Dublin, CA); a single injection hole made with the 30g needle, which was inserted 3 mm into the vitreous cavity; and pirenzepine or PBS injected. The same injection was measured with digital vernier calipers, and the results were expressed as the differences between the treated and control eyes.

**Induction of Refractive Error**

Induction of refractive error was achieved by using polymethylmethacrylate (PMMA) lenses (base curve, 7 mm; total diameter, 12.5 mm) with powers of +10 and −10 D (Australian Contact Lenses, Carlton, Victoria, Australia), placed in front of the treated eye by means of a goggle fashioned from Velcro. The calculated effective powers at the corneal plane were +10.5 and −9.5 D, since the posterior lens surfaces were 5 mm in front of the corneal plane of the eye: \( F_{\text{eff}} = F_{\text{opt}}/1 - d/F_{\text{opt}} \), where \( F_{\text{opt}} \) is the effective lens power at the cornea, \( d \) refers to the distance in meters between the corneal plane and the posterior lens surface, and \( F_{\text{opt}} \) is the optical power of the PMMA lens used. However, they will be referred to as the +10 or −10 D groups on the basis of the optical rather than the effective lens powers. The lenses were cleaned daily.

**Sulfate Labeling of Scleral GAGs**

To obtain a metabolic correlate of any ocular growth changes, we measured glycosaminoglycan (GAG) synthesis in the posterior sclera (8 mm punch). On the final experimental day (fourth day), 1 hour after the final intravitreal injection or sham injection, each chick was injected intraperitoneally with 100 μCi of radiolabeled sulfate (\(^{35}\text{SO}_4\)) in a volume of 200 μL. The animals were then returned to the brooder for a further 2 hours of visual experience before the eyes were measured by retinoscopy and A-scan ultrasound, and tissue was taken for analysis.

**Ocular Measurements**

On the final (fourth) experimental day, 3 hours after the last intravitreal injection, the chicks were anesthetized with 75 mg/kg ketamine (100 mg/mL) and 5 mg/kg xylazine (20 mg/mL), and body temperature was maintained with a heating pad. A dental bite bar was taped to the beak of the chick fixed in a universal joint, to enable accurate orientation of the head to allow optical and biometric data to be collected on the central axis of the eye. Ocular measurements, namely refraction and axial dimensions were collected by using retinoscopy and A-scan ultrasound with a 10-MHz transducer, as described elsewhere. Axial length refers to the internal axial length, defined as the sum of the anterior chamber depth (front of cornea to front of crystalline lens), lens thickness, and the vitreous chamber depth (back of crystalline lens to front of vitreoretinal interface). Each recorded value of axial biometric measurements was the mean of six averaged waveforms (each in itself an average of 20 incoming waveforms). The results were expressed as the mean of final values for the six animals in each group or the average difference between the values for the treated and control eyes within a group.

**Enucleation and Equatorial Diameter Measurements**

After all in vivo measurements, the chicks were given a lethal dose (325 mg/kg) of pentobarbitone sodium (325 mg/mL) intraperitoneally. The eyeball was enucleated after the 12 o’clock position was marked. Once the eye was enucleated, the mean equatorial diameter (\( H + V \))/2 was measured with digital vernier calipers, and the results were expressed as the differences between the treated and control eyes.

**Tissue Dissection and Measurement of GAG Synthesis**

After equatorial diameter measurements, the eye was separated into anterior and posterior halves with a razor blade. An 8-mm diameter surgical trephine was used to punch a button out of the posterior segment of the eyes, which included the posterior pole but avoided the pecten and optic nerve head. The retina and choroid were carefully removed from the sclera. The posterior scleral tissue punch was then snap frozen in liquid nitrogen. The tissues were stored at −80°C until assayed.

The tissue samples were freeze-dried overnight (MicroModulyo; Edwards, Crawley, UK). Dry weights were measured on a microbalance (R200D; Satorius, AG, Göttingen, Germany) to the nearest 0.001
mg. Scleral tissue was then digested as previously described, and the digested scleral tissue samples were stored at −80°C until assayed.

GAG incorporation of the radiolabel was assessed by selective precipitation, with a modified version of the Alcian blue dye–binding method. Details of the assay, as used on chick sclera, have been published.

Sulfate incorporation was measured as counts per minute in 10 mL of scintillation solution (Cytosprint; Fisher Scientific, Scoresby, VIC, Australia) over a 10-minute period with a liquid scintillation counter (Wallac 1409; Perkin Elmer, Boston, MA). All tissue samples were analyzed in triplicate and the results expressed as decays per minute after correction for quenching effects by using an external standard. The final results were expressed as the percentage difference in the incorporation of sulfate into GAGs in the treated and control eyes and normalized to both dry weight and DNA content. DNA content was measured with the Hoechst 33258 assay (Sigma-Aldrich, Castle Hill, NSW, Australia), as described elsewhere.

Statistical Analyses

Paired Student’s t-tests were used to compare treated and control eyes of the same animal (all analysis by GraphPad Software, Inc., San Diego, CA). Unpaired tests were used to compare either the absolute treated control eye differences or the percentage differences between different groups. A one-way ANOVA with Tukey’s multiple comparison test was used for comparisons involving more than two groups of animals. All results are presented as the mean ± SEM, unless otherwise stated.

RESULTS

Refractive and Ocular Biometry

To confirm that differences in measurements of treated versus control eyes were due only to treatment-induced changes, we compared the control eye data sets of the eight groups, across the nine experimental measurements. Since statistical analysis (ANOVA) revealed no significant differences in any parameter among the fellow control eyes in any of the eight experimental groups, the treated–control eye differences indicate only changes induced in the treated eye. Therefore, in the sections that follow, the changes in all parameters in the treated eye are expressed as differences (treated minus control).

Treated eyes in the +10 D group were significantly hyperopic relative to the contralateral control eyes (+15.9 ± 0.8 D vs. +4.0 ± 0.3 D, P < 0.001), giving a treated–control eye difference of +9.9 ± 0.5 D (Fig. 1A). This equated to 94% compensation for the effective lens power (+10.5 D). The treated eyes in the −10 D group were significantly myopic compared with the control eyes (−5.8 ± 1.3 D vs. +2.9 ± 0.4 D, P < 0.001), with a difference between treated and control eyes of −8.7 ± 1.3 D (Fig. 1A). This difference equated to 92% compensation for the effective lens power (−9.5 D). Treated eyes in the +10 D group had shallower vitreous chambers (−0.39 ± 0.04 mm, P < 0.001, Fig 1B) than did the contralateral control eyes, whereas treated eyes in the −10 D group had deeper vitreous chambers than did the control eyes (+0.20 ± 0.05 mm, P < 0.01, Fig 1B). The axial length changes were in

The vitreous chamber depth changes observed in binocularly open animals were not significantly different. (C) Differences in ocular axial length between treated and contralateral control eyes in the experimental groups: The vitreous chamber was the major contributor to the changes observed in the ocular axial length. (A-C) Asterisks not associated with brackets denote P-values from comparisons between treated and control eyes in a single group, and those associated with brackets between bars are from comparisons of treated minus control differences between the two groups. *P < 0.05, **P < 0.01, ***P < 0.001, n.s. represents P > 0.05. Error bars, 1 SEM; n = 6, all groups.
the same direction as the vitreous chamber depth changes (Fig. 1C). The axial change in the vitreous chamber depths accounted for the main structural correlate to the induced refractive error. Animals in the −10 D group were found to have a significantly enlarged mean equatorial diameter in the treated eyes (11.74 ± 0.40 mm vs. 11.57 ± 0.43 mm, P < 0.01; Fig 2), whereas there were no significant differences in mean equatorial diameter between the treated and control eyes in the +10 D group (11.71 ± 0.27 mm vs. 11.68 ± 0.16 mm, P > 0.05, Fig 2). These results are comparable to data from an earlier study on lens-induced refractive errors in chicks.22

Daily pirenzepine injections (700 μg) were effective in inhibiting nearly all the myopia induced by negative lenses in the −10 D-sal group when compared with the −10 D-sal group (−1.1 ± 1.5 D vs. −8.8 ± 1.1 D, P < 0.01, Fig. 1A). The interocular vitreous chamber depth difference in the −10 D-pz group was significantly less than in the −10 D-sal group (−0.03 ± 0.04 mm vs. +0.26 ± 0.06 mm, P < 0.01, Fig. 1B). Pirenzepine treatment also caused a significant reduction in the anterior chamber depth in the treated eyes of the −10 D-pz group compared with that in the control eyes (1.26 ± 0.08 mm vs. 1.40 ± 0.09 mm, P = 0.01; Table 1). This relative decrease in anterior chamber depth was significant when compared with that in the −10 D-saline group (−0.14 ± 0.04 mm vs. 0.02 ± 0.04 mm, P < 0.01; Table 1). The equatorial diameter changes in the −10 D-pz group were not significantly different from those in the −10 D-sal and the −10 D groups (ANOVA, P > 0.05).

Daily pirenzepine injections (700 μg) did not significantly alter the refractive changes induced by positive lenses. The interocular difference in refractive error in the +10 D-pz group was not significantly different from that in the +10 D-sal group (+8.1 ± 0.6 D vs. +8.9 ± 2.4 D, P = 0.76; Fig. 1A). The changes in interocular vitreous chamber depth were not significantly different between the +10 D-pz and the +10 D-sal groups (−0.44 ± 0.05 mm vs. −0.49 ± 0.10 mm, P = 0.66, Fig. 1B), in keeping with the refractive findings. The relative changes in equatorial diameter did not differ significantly between the +10 D-pz, +10 D-sal, and +10 D groups (ANOVA, P > 0.05). There were no differences in the induced refractive error or ocular growth changes between the +10 D-sal and the +10 D groups or between the −10 D-sal and the −10 D groups (P >

![Figure 2](http://iovs.arvojournals.org/pdfaccess.ashx?url=/data/journals/iovs/932968/)

**TABLE 1.** Summary of Ocular Refractive and Structural Data in Experimental Groups Collected on the Fourth Treatment Day

<table>
<thead>
<tr>
<th>Group</th>
<th>Effective refraction, D Mean (SD)</th>
<th>Anterior chamber, mm Mean (SD)</th>
<th>Lens thickness, mm Mean (SD)</th>
<th>Vitreous chamber, mm Mean (SD)</th>
<th>Axial length, mm Mean (SD)</th>
<th>Equatorial diameter, mm Mean (SD)</th>
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<td>−10 D</td>
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<td>+10 D-sal</td>
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<td>Open-pz</td>
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<tr>
<td>Open-sal</td>
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0.05), suggesting that the daily injection procedure did not induce significant structural changes per se.

Chicks with no lens defocus that were treated with pirenzepine (open-pz) developed significant hyperopia in the treated eyes compared with that in the contralateral control eyes (+5.3 ± 1.5 D, P < 0.01, Fig. 1A), which was due to significant shortening of the vitreous chamber depth in the treated eyes (−0.24 ± 0.08 mm, P < 0.05, Fig. 1B). The open-pz group was significantly hyperopic compared with the open-sal group (+5.3 ± 1.5 D vs. +1.6 ± 1.2 D, P < 0.05, Fig. 1A), indicating that pirenzepine alone caused relative hyperopia. The vitreous chamber depth differences between the open groups were not statistically significant, although the changes were in the direction that explained the refractive effects (−0.24 ± 0.08 mm vs. −0.17 ± 0.04 mm, P = 0.22; Fig. 1B). A nonsignificant difference in the axial length was observed between the open-pz group and the open-sal group (−0.31 ± 0.07 mm vs. −0.18 ± 0.04 mm, P = 0.07). Equatorial dimensions in the treated eyes of binocularly open groups (open-pz and open-sal groups) were not significantly different from the contralateral control eyes (P > 0.05), and there were no differences between these groups (P > 0.05).

GAG Synthesis

Radiolabeled sulfate incorporation (DPM/µg DNA) in the posterior sclera was not significantly different between eyes treated with positive lenses (+10 D) when compared with the contralateral untreated control eyes (−2.4% ± 9.0%, P > 0.05, Fig 3), whereas eyes treated with negative lenses (−10 D) showed significant increases compared with the contralateral control eyes (61.2% ± 23.5%, P < 0.05, Fig. 3). There was no significant difference between the three +10 D positive-lens–treated groups in the reduction in sulfate incorporation (one-way ANOVA, P > 0.05), and the reduction was only significantly different from 0 in the +10 D-pz group (−31.6% ± 8.6%, P < 0.01, Fig. 3). Chick eyes treated with −10 D negative lenses all showed an increase in sulfate incorporation when compared with the contralateral untreated control eyes (Fig. 3). There was no significant difference between the three −10 D negative-lens–treated groups in the increase in sulfate incorporation (one-way ANOVA, P > 0.05), and these increases were significantly different from 0 (𝑡-test, P < 0.05) in all −10 D negative-lens–treated groups (Fig 3).

No significant difference in sulfate incorporation was observed between the open-pz and open-sal groups (16.3% ± 15.2% vs. −26.1% ± 9.7%, P > 0.05; Fig 3) and the mean difference in sulfate incorporation was not significantly different from 0 for both groups (𝑡-test, P > 0.05).

No significant differences were observed in the DNA content of the posterior scleral tissue button between treated and control eyes in the eight groups or in the dry tissue weight differences of each button (one-way ANOVA, P > 0.05, data not shown). Consequently, when GAG synthesis was normalized to milligrams dry tissue weight (decays per minute per milligram dry weight; data not shown) instead of DNA content, no differences were found from results expressed as sulfate incorporation per microgram of DNA (decays per minute per milligram DNA).

DISCUSSION

The principal finding of this study is that, at the minimum dose (700 µg) demonstrated to be effective in preventing form-deprivation–induced axial myopia in the chick, daily pirenzepine did not alter the magnitude of positive-lens (+10 D) induced hyperopia. Specifically daily pirenzepine treatment neither enhanced the amount of induced hyperopia, nor inhibited it, contrary to proposed hypotheses. In addition, daily pirenzepine treatment (700 µg) prevented nearly all negative-lens (−10 D)–induced axial myopia, which is the first demonstration of inhibition of negative-lens–induced myopia in the chick model using nontoxic doses of pirenzepine.

Pirenzepine has been reported to prevent negative-lens–induced myopia and axial ocular elongation in mammalian animal models, including tree shrew11 and guinea pig.14 However, pirenzepine did not affect the relative equatorial enlargement during negative-lens–induced myopia (Fig 2), contrary to the findings of the effect of the broad-band muscarinic antagonist atropine on form-deprivation myopia,5 which may relate to the stronger relative affinity of atropine for all muscarinic receptors.

All three groups of chicks treated with −10 D lenses had significant increases in GAG synthesis in the posterior sclera of the treated eye, relative to the contralateral control eyes, but there was no significant difference between the three groups, even though the −10 D pirenzepine–treated chicks developed significantly less myopia and elongation of the vitreous chamber. A previous study reported that pirenzepine transiently prevented the increased GAG synthesis after 2 hours in form-deprived chicks.16 This same study also demonstrated that a similar transient decrease in GAG synthesis was observed when form-deprivation was interrupted with 3 hours of unoccluded vision, which also prevents myopia development. These findings demonstrate that a transient decrease in GAG synthesis can produce a sustained decrease in the rate of myopia development, whether produced by the physiological regulation of ocular growth of unoccluded vision or a pharmacologic regulation of ocular growth with pirenzepine.18 The lack of a transient decrease in GAG synthesis in the present study was most likely due to the known differences in the time-course of ocular growth responses of chicks to negative-lens defocus and to diffusers.23 Negative-lens defocus induces a more rapid elongation than diffusers and thus assessment at
the same time period in both studies (2 hours after labeling) was probably too late to assess the transient inhibition of GAG synthesis in the sclera.

Although the exact mechanism by which pirenzepine causes this reduction in myopia and ocular elongation has not yet been elucidated, pirenzepine's effects are unlikely to be mediated by toxicity. No retinal or scleral toxic effects have been reported with the doses of pirenzepine (500–700 µg) used during form-deprivation studies in chicks and tree shrews. In these studies, pirenzepine did not alter retinal and scleral tissue morphology, DNA content, or the ability of pirenzepine-treated eyes to become myopic after pirenzepine treatment was discontinued.

Pirenzepine did not enhance or attenuate the hyperopia and axial ocular growth changes induced by positive lenses alone (Fig. 1A). We had hypothesized that combining positive-lens defocus and pirenzepine would enhance the amount of hyperopia induced, as pirenzepine not only antagonizes myopia development, but also induces a small amount hyperopia in eyes with no induced defocus. The most likely explanation of why this did not occur is that the functional emmetropization mechanism inherent in the eye prevented further shortening of the vitreous chamber once full compensation for the induced optical defocus was reached (94% compensation for the +10 D defocus used in this study) producing a clear image on the retina. Previous studies have demonstrated that animal models of refractive error functionally emmetropize to the degree of induced defocus and no more. Specifically how a clear retinal image stabilizes ocular growth and refractive state, whether it is overriding a pharmacologic intervention such as pirenzepine or is a recovery response with optical correction of the induced myopia is not known, other than that it is likely to be related to peak firing of specific retinal neurons that sense image clarity and feed into regulation of ocular growth (e.g., amacrine cells).

In addition, there was no evidence to indicate that the speed of compensation in response to the induced defocus was faster in the eyes treated with a combination of positive-lens defocus and pirenzepine. While full refractive and ocular biometry measurements were recorded only on the final day of the experiment, retinoscopy was performed before every intravitreal injection, first to confirm that the ocular media were clear and no interocular injury or infection had occurred due to injections and second to perform an approximate assessment of the refractive error of the treated eye by noting the direction and speed of movement of the retinoscopic reflex. No marked differences were noted in the rate of compensation with +10 D lenses between the three groups of chicks treated with +10 D lenses. This conclusion is further supported by finding that all three groups of +10 D-treated chicks had similar reductions in GAG synthesis in the posterior sclera, indicating similar metabolic rates of scleral remodeling.

The possibility that pirenzepine may simply attenuate any deviation from normal ocular growth, whether positive- or negative-lens induced was also considered. It has been reported that the hyperopic shift induced by positive-lens defocus is due to the anterior displacement of the retina, in which a major contributor is choroidal thickening, whereas, the myopic shift induced by negative lens defocus is predominantly due to elongation of the vitreous chamber depth, to which choroidal thinning makes only a minor contribution. The significantly thicker choroid in positive-lens-defocus–treated eyes may impede the physical diffusion of pirenzepine to sites in the choroid or sclera that could be involved in cholinergic control of ocular growth. At present, the active site of cholinergic inhibition of ocular growth has not been clearly elucidated, and although the retina seems the most likely location, muscarinic receptors have been identified in both the choroid and the sclera. Thus, the access of pirenzepine to scleral or choroidal sites may be important.

Another possible reason that intravitreally injected pirenzepine was ineffective in preventing positive-lens-induced hyperopia could be that the sustained action of positive defocus (all day) exceeded the duration of pirenzepine’s bioactivity in ocular tissues. Although the positive lens signal has been shown to be resistant to 2 hours of nonlens visual exposure daily, this visual exposure may be a more powerful signal than pirenzepine (half-life of 4 hours in the retina) (Yin GC, et al. IOVS 2003;44:ARVO E-Abstract 4339). Thus, pirenzepine’s effect on ocular growth is overridden by the sustained effect of positive-lens defocus.

Although pirenzepine is classed as an M₄-selective muscarinic antagonist, the chick model does not possess an M₁-receptor (Yin GC, et al. IOVS 2003;44:ARVO E-Abstract 4339), which raises questions about the specific receptor-based mechanism by which pirenzepine mediates ocular growth. However, the binding affinity of pirenzepine to the mammalian M₁-receptor is only four times lower than for the mammalian M₁-receptor, and the affinity of pirenzepine for the chick M₂-receptor (M₁ surrogate in the chick) is nearly the same as its affinity for the M₄-receptor. Thus, pirenzepine may mediate its effects through the M₄ or M₂-receptor (Yin GC, et al. IOVS 2003;44:ARVO E-Abstract 4339). More recently, the highly selective M₄ antagonist MT3 has been shown to be effective in reducing experimentally induced myopia and axial elongation in chicks (Morgan IG, et al. IOVS 2005;46:ARVO E-Abstract 3342; Diether S, et al. IOVS 2005;46:ARVO E-Abstract 1986; McBrien NA, et al. IOVS 2009;50:ARVO E-Abstract 3850). Although the M₄-receptor pathway seems most likely, recently it has been reported (Diether S, et al. IOVS 2005;46:ARVO E-Abstract 1986; McBrien NA, et al. IOVS 2009;50:ARVO E-Abstract 3850) that the highly selective M₄ muscarinic antagonist MT is partially effective in reducing experimental myopia in the chick model of refractive development. This finding could be interpreted to indicate that multiple muscarinic receptors are involved in the regulation of ocular growth and myopia, although the highly selective nature of MT3 and -7 have yet to be confirmed with binding assays; thus, nonspecific effects cannot be ruled out.

In summary, combining pirenzepine treatment with positive lens defocus did not alter the amount of induced hyperopia nor the reduction in the rate of GAG synthesis caused by positive-lens–induced hyperopia. We propose that compensatory eye growth under imposed myopic defocus rapidly produces a clear retinal image, which prevents pirenzepine from causing a further hyperopic shift. In contrast, pirenzepine prevented the development of induced myopia due to negative lens treatment (hyperopic defocus). We propose that because changes in ocular growth are slower to achieve compensation during imposed hyperopic defocus than during myopic defocus, more time is available for pirenzepine to exert its inhibitory effect on the development of myopia.

References


