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Daily or Less Frequent Topical 1% Atropine Slows Defocus-Induced Myopia Progression in Contact Lens-Wearing Guinea Pigs

Qiurong Zhu[1,2], So Goto[1,3,4], Sarah Singh[1], Josue A. Torres[1], and Christine F. Wildsoet[1]

[1] Herbert Wertheim School Optometry and Vision Science, University of California, Berkeley, California, USA
[2] Department of Optometry and Visual Science, West China Hospital of Sichuan University, Sichuan, China
[3] Department of Ophthalmology, Osaka University Graduate School of Medicine, Osaka, Japan
[4] Laboratory for Retinal Regeneration, RIKEN Center for Biosystems Dynamics Research, Kobe, Japan

Correspondence: Christine F. Wildsoet, Herbert Wertheim School Optometry and Vision Science, University of California, Berkeley, CA 94720, USA. e-mail: wildsoet@berkeley.edu

Purpose: This study compared the efficacy of topical 1% atropine applied daily versus every 3 days for controlling myopia progression in guinea pigs.

Methods: To induce myopia, pigmented guinea pigs (New Zealand strain, $n = 38$) wore monocular -10 D rigid gas-permeable (RGP) contact lenses, which were replaced after 3 weeks with -15 diopter (D) contact lenses. Animals were treated with 1% atropine either daily (Atr-QD; $n = 12$), or every 3 days (Atr-Q3D; $n = 11$), or with artificial tears (control group; $n = 15$). Spherical equivalent refractive error (SER) and axial length (AL) data, as well as retinal and choroidal thickness data were collected weekly.

Results: Whereas mean ($\pm$SEM) interocular differences (treated - fellow) in both SER and AL at week 0 (baseline) were similar for all groups, significant differences between the atropine-treated and control groups were evident by week 6 (SER and AL, $P < 0.001$). The treated eyes of the control group showed relatively more axial elongation and myopia progression than both the Atr-QD and Atr-Q3D groups. Choroidal blood vessel area also decreased over time in the treated eyes of the control group, coupled with choroidal thinning overall, with these changes being attenuated by atropine. Retinal thickness showed a developmental decrease over the treatment period but was unaffected by atropine.

Conclusions: For this defocus-induced guinea pig model of myopia, application of 1% topical atropine slows myopia progression, even when applied every 3 days.

Translational Relevance: The results from this study suggest that the frequency of dosing for topical atropine may be reduced from the widely used daily dosing regimen without loss of myopia control efficacy.

Introduction

Myopia, or near-sightedness, reflects a mismatch between the axial length of the eye and its refractive power, typically due to excessive ocular elongation. This causes parallel light rays from distant objects to focus in front of the retina, resulting in blurred vision without correction. Myopia is now the most common refractive error worldwide.[1] The prevalence of myopia has also increased rapidly in recent decades, especially in East Asia, where 80% to 90% of young adults are myopic.[2] In the United States, the preva-lence of myopia is approximately 44%, slightly higher than its prevalence in Europe of 30%.[3,4] While non-cycloplegia refraction data may lead to an overestima-tion of myopia prevalence, especially in studies involv-ing children, that it is increasing worldwide is beyond doubt. It has been predicted that there will be nearly 5 billion people with myopia and 1 billion people with high myopia worldwide by 2050, accounting for 50% and 10% of the global population, respectively.[5] Myopia is also associated with irreversible vision-threatening complications, including choroidal neovas-cularization, myopic maculopathy, and retinal detach-ment, with high myopes being at greatest risk.[6] For

these various reasons, myopia has become a major global public health concern, with significant research efforts now devoted to the development of effective methods for delaying myopia onset or slowing its progression.

Among the currently used myopia control treatments, two anti-muscarinic pharmaceutical agents, atropine and pirenzepine, applied topically, have been shown to be effective in slowing myopia progression.[7,8] Atropine is a nonselective muscarinic acetylcholine receptor antagonist that was first shown to be effective in controlling myopia progression in the 1960s, administered as a daily topical 1% formulation.[9] A number of more recent clinical trials have shown that this effect of atropine is also dose dependent. For example, results from the ATOM1 and ATOM2 studies showed increasing treatment efficacy with increasing dose for 4 atropine concentrations, 1%, 0.5%, 0.1%, and 0.01%, which reduced myopia progression by approximately 80%, 75%, 70%, and 60%, respectively, over a 2-year period, compared to progression in the placebo group.[10,11] Likewise, the more recent LAMP study reported decreases in myopia progression of 67%, 43%, and 27%, and in axial length elongation of 51%, 29%, and 12% after 1 year for 0.05%, 0.025%, and 0.01% atropine concentrations, respectively.[12] All three studies involved daily topical applications.

Although topical ophthalmic atropine is now used widely clinically for slowing myopia progression, either as an approved or off-label treatment, several studies have reported dose-dependent side-effects, including photophobia, glare, blur, and allergic reactions, which have proved to be intolerable for some participants.[13] Note that in clinical trials, dose-dependent attenuation of treatment efficacy and rebound effects after termination of atropine treatments (i.e. accelerated myopia progression), have also been reported. For example, in the ATOM studies, more than 0.50 D myopia progression was observed in 68%, 59%, and 24% of children treated with 0.5%, 0.1%, and 0.01%, respectively, during a 1-year washout phase.[10,11] As treatments to reduce myopia progression are generally initiated in early childhood (between the age of 6 and 12 years), with a likely need for treatments extending over many years, it is important to minimize exposure, while not compromising treatment efficacy. Given the well-recognized long duration of action of topical atropine, when applied to the eye for diagnostic purposes, the possibility of reducing exposure through less frequent dosing, while maintaining treatment efficacy, as examined in the study reported here, was deemed plausible.[14,15]

Animal model studies have provided critical insights into the mechanisms underlying refractive error development and myopia, and have also provided valuable data on various therapeutic interventions. The guinea pig has become an important and widely used mammalian myopia model for this purpose, offering a number of advantages, including its relatively large eye size and cooperative nature.[16] Similar to other myopia animal models, guinea pigs respond to both negative lens-imposed defocus and form deprivation conditions to become myopic.[17,18] For studies involving atropine in particular, guinea pigs share several relevant characteristics with human eyes. Muscarinic receptors are present throughout its ocular tissues, including the retina, choroid, and sclera.[19] As in humans, their iris and ciliary muscles are made up of smooth muscle fibers, with muscarinic receptors,[20] contrasting with the equivalent muscles in the chick eye, which are comprised of skeletal muscle fibers with nicotinic receptors.[21,22]

This study investigated the anti-myopia effects of topical 1% atropine sulfate in pigmented guinea pigs, with specific interest in the comparative efficacy for myopia control of topical 1% atropine applied daily versus every 3 days. The study design made use of our recently described contact lens model for myopia. As detailed in the following sections, reducing the atropine dosing frequency to once every 3 days had minimal effect on treatment efficacy.

Materials and Methods

Animals

Two-week-old New Zealand strain pigmented guinea pigs were used in this study and bred on-site. Pups were weaned at 5 days of age and reared as single-sex pairs in transparent plastic tubs. Animals were housed in a temperature-controlled room with a 12-hour light/12-hour dark cycle (on at 9:30 AM and off at 9:30 PM). They had free access to water and vitamin C-supplemented food, and received fresh vegetables and fruits three times a week as dietary enrichment. All animal care and treatments used in this study conformed to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Experimental protocols were approved by the Animal Care and Use Committee of the University of California, Berkeley.

Rigid Gas-permeable Contact Lenses

In total, 38 guinea pigs wore monocular −10 D rigid gas permeable (RGP) contact lenses for 3 weeks, when they were replaced with −15 D RGP contact lenses, which were worn for a further 3 weeks, yielding a total treatment period of 6 weeks. The contact lenses (Valley

Contax, Springfield, OR, USA) were made from acrylic fluorosilicone material, which has a high oxygen permeability (65%), and custom-designed, with an overall diameter of 6.00 mm, optic zone diameter of 5.00 mm, and base curve of 3.38 mm. Details concerning lens wear and monitoring schedules are described in a previous publication.[23] In brief, the contact lenses were worn continuously and checked three times a day, with each lens being removed and replaced with a clean lens every morning. Lenses were rinsed thoroughly with Opti-Free soft contact lens solution (Alcon, Fort Worth, TX, USA), prior to insertion. While not in use, lenses were soaked in a combination of Boston protein remover and Boston Simplus solution (Bausch and Lomb, Rochester, NY, USA).

Topical Atropine Sulfate Treatments

Topical treatments were confined to the contact lens-wearing eyes of the guinea pigs, delivered at 10:00 AM, when the contact lenses were also replaced. Twelve animals were treated each day with one drop of 1% atropine (Akorn, Lake Forest, IL, USA; Atr-QD), whereas 11 animals received one drop of 1% atropine every 3 days (Atr-Q3D). Another 15 animals received topical artificial tears (Johnson & Johnson, New Brunswick, NJ, USA), as a placebo control treatment (control group). The fellow untreated eyes of all animals served as contralateral controls for their respective groups.

Measurements

Baseline refractive error and axial length data, as well as anterior corneal curvature, retinal thickness, and choroidal thickness data were collected and then at weekly intervals after initiation of treatments. From collected high resolution choroidal images, various structural parameters were also derived. Measurements on individual animals were performed at the same time of day, around 1:00 PM, to avoid possible confounding effects of circadian rhythms in eye growth.

Both refractive error and axial length data were collected on awake animals. Refractive errors were measured using streak retinoscopy (Welch Allyn, Skaneateles Falls, NY, USA), following cycloplegia with 1% cyclopentolate hydrochloride (Bausch & Lomb, Rochester, NY, USA), instilled 30 minutes prior to measurement, and are reported as spherical equivalent refractive errors (SERs; average of the results for the two principal meridians). A Lenstar (Haag-Streit Holdings, Köniz, Switzerland) was used to measure axial lengths (ALs). For these measurements, the Lenstar chin rest was replaced with a platform on which the guinea pigs were seated for measure-

ments. Each measurement comprised an average of at least three readings. The Lenstar output includes peaks representing the anterior and posterior corneal surfaces, the front and back of the crystalline lens, vitreous/retina interface, and retina/choroid interface. The distances between these various peaks represent the following five ocular axial dimensions: central corneal thickness (CCT), anterior chamber depth (ACD), lens thickness (LT), vitreous chamber depth (VCD), and retinal thickness (RT). The AL reported here refers to the distance from the anterior surface of the cornea to the posterior surface of the retina (the retina/choroid interface), commonly referred to as the optical AL.

Corneal curvatures were derived from high-resolution images of the anterior ocular segment captured with a Visante Anterior Segment Optical Coherence Tomography (AS-OCT; Zeiss Meditec, Dublin, CA, USA). Guinea pigs were also not anesthetized for AS-OCT imaging. For optimal imaging, the position of each guinea pig was adjusted to ensure the images to be scanned were clear and in the center of the screen. Observation of a bright white line running perpendicular to the corneal apex was used as an indicator of good alignment and high-quality imaging. Captured images were processed off-line using a customized MATLAB program (MathWorks, Natick, MA, USA). In brief, both the right and left limbal margins (boundary between white sclera and grainy cornea) were first identified and a circle connecting these two points and the anterior corneal apex generated, the latter being identified automatically by the software. The radius of the circle was taken as the anterior corneal radius of curvature (CRC). Although this method can also be applied to obtain the posterior CRC, only anterior CRCs were analyzed in this study.

Spectral-domain optical coherence tomography (SD-OCT; Bioptigen Envisu R-Series, Morrisville, NC, USA) was used to image the posterior ocular layers and so to obtain choroidal thickness (ChT) and retinal thickness (RT) data, as well as choroidal structural details (vascular and total interstitial areas). Guinea pigs were first anesthetized with a ketamine/xylazine cocktail (27/0.6 mg/kg body weight), and then positioned on a customized platform for imaging. The SD-OCT scanning protocol used in this study was as previously described[24] (i.e. 70 B-scans and 700 A-scans, with 30 frames per B-scan and a 2.6 × 2.6-mm-wide field of view). Choroidal analyses were restricted to the visual streak region, which is approximately 2.5 optic nerve head (ONH) diameters (approximately 700 μm) away from the center of the ONH. The use of the ONH as a reference landmark allowed capture of cross-sectional images from the

same ocular fundus area at each measurement time point. The cornea was massaged through the eyelids, at approximately 5-minute intervals during imaging, to maintain the integrity of the tear film, in the interest of good quality images.

The built-in calipers in the Bioptgen instrument were used to measure ChT and RT from captured cross-sectional images. The middle third of the cross-sectional images was selected for analysis to avoid optical distortions affecting more peripheral (off-axis) parts of images. Choroidal vascular luminal and interstitial areas were also determined as described previously, using the binarization method in the Image J software (http://imagej.nih.gov/ij/; provided in the public domain by the National Institutes of Health, Bethesda, MD, USA).[25] In brief, the middle third of each SD-OCT image was selected and added in the ImageJ ROI manager. Luminal areas were determined using the Threshold Tool after image manipulations aimed at reducing the noise in images. In addition to total luminal areas, total interstitial and choroidal areas were calculated, and the ratio of total vessel area to total choroidal area subsequently derived.

In a pilot study, a NeurOptics PLR-3000 pupillometer (NeurOptics, Irvine, CA, USA), modified with the attachment of a +16 D auxiliary imaging lens, was used to investigate the mydriatic effect of 1% topical atropine in guinea pigs. Adult guinea pigs were used in this study, because of the difficulty in reliably identifying the iris pupil boundary in young guinea pigs. Measurements were made on awake animals under a room illumination of 160 to 180 lux. The maximum resting pupil diameter (mm), minimum (contracted) pupil diameter (mm) after light stimulation, and the contraction ratio (%) were recorded. To establish the duration of action of one drop of 1% topical atropine in the guinea pig eye, data were collected from 4 guinea pigs, whose pupils were tracked over 5 days after instillation, measurements being made 1 hour later, and then daily up to 5 days. Additional data were obtained from nine guinea pigs, which were treated in one eye with daily topical 1% atropine for 6 weeks and monitored at weekly intervals.

Statistical Analysis

All the data were analyzed using Prism 7 (GraphPad Software, La Jolla, CA, USA). Results for treated and control (fellow) eyes, as well as the interocular differences (treated eye − fellow eye) differences, are reported as mean ± SEM. Mixed-model repeated measures ANOVAs with a Bonferroni post hoc test were used to compare treated eyes and control eyes within each group, as well as changes over time in interocular differences across the three groups, for various ocular parameters. Any *P* values less than 0.05 were considered to be significant. Regression analyses were performed to evaluate the relationship between interocular differences in AL and VCD at the end of the study, for each of the three groups.

Results

Before the initiation of treatments, interocular differences in all measured parameters, including SER, AL, CRC, RT, and ChT were minimal for all three groups (Table). Thus statistically, there were no significant differences between treated and fellow (contralateral control) eyes within each group at this time.

Effects of Atropine on Myopia Progression and Ocular Axial Dimensions

Refractive errors (SERs): As expected, the contact lens-wearing eyes of the control group elongated significantly more than their fellows over the treatment period, to become myopic by the end of the study. Interocular differences in SER had already increased

Table. Summary of Baseline Refractive Error and Ocular Biometric Parameters for the Three Groups (Mean ± SEM)

Groups and Eyes	Control Group		Atr-QD		Atr-Q3D	
	Right Eyes	Left Eyes	Right Eyes	Left Eyes	Right Eyes	Left Eyes
Spherical equivalent refraction, D	4.13 ± 0.50	4.03 ± 0.46	4.83 ± 0.54	4.13 ± 0.65	3.66 ± 0.38	3.57 ± 0.42
Axial length, mm	6.91 ± 0.03	6.94 ± 0.04	6.94 ± 0.04	6.93 ± 0.04	6.98 ± 0.03	7.00 ± 0.04
Curvature radius of the cornea, mm	3.45 ± 0.03	3.48 ± 0.04	3.51 ± 0.03	3.57 ± 0.05	3.54 ± 0.04	3.58 ± 0.04
Retinal thickness, μm	150.71 ± 0.72	151.64 ± 1.10	151.36 ± 0.80	151.64 ± 0.76	151.44 ± 0.59	152.49 ± 0.53
*Choroidal thickness, μm	85.39 ± 2.90	84.13 ± 2.86	79.27 ± 3.07	81.22 ± 2.62	81.71 ± 3.19	84.40 ± 2.94
*Total choroidal luminal area, μm²	27,380.20 ± 1362.06	27,020.10 ± 1090.87	25,597.52 ± 1551.20	25,882.47 ± 4025.62	25,224.70 ± 1411.47	28,306.21 ± 1464.15

*Derived for middle third of captured SD-OCT images.
No significant differences in ocular parameters between the right eyes and left eyes within each group at baseline.
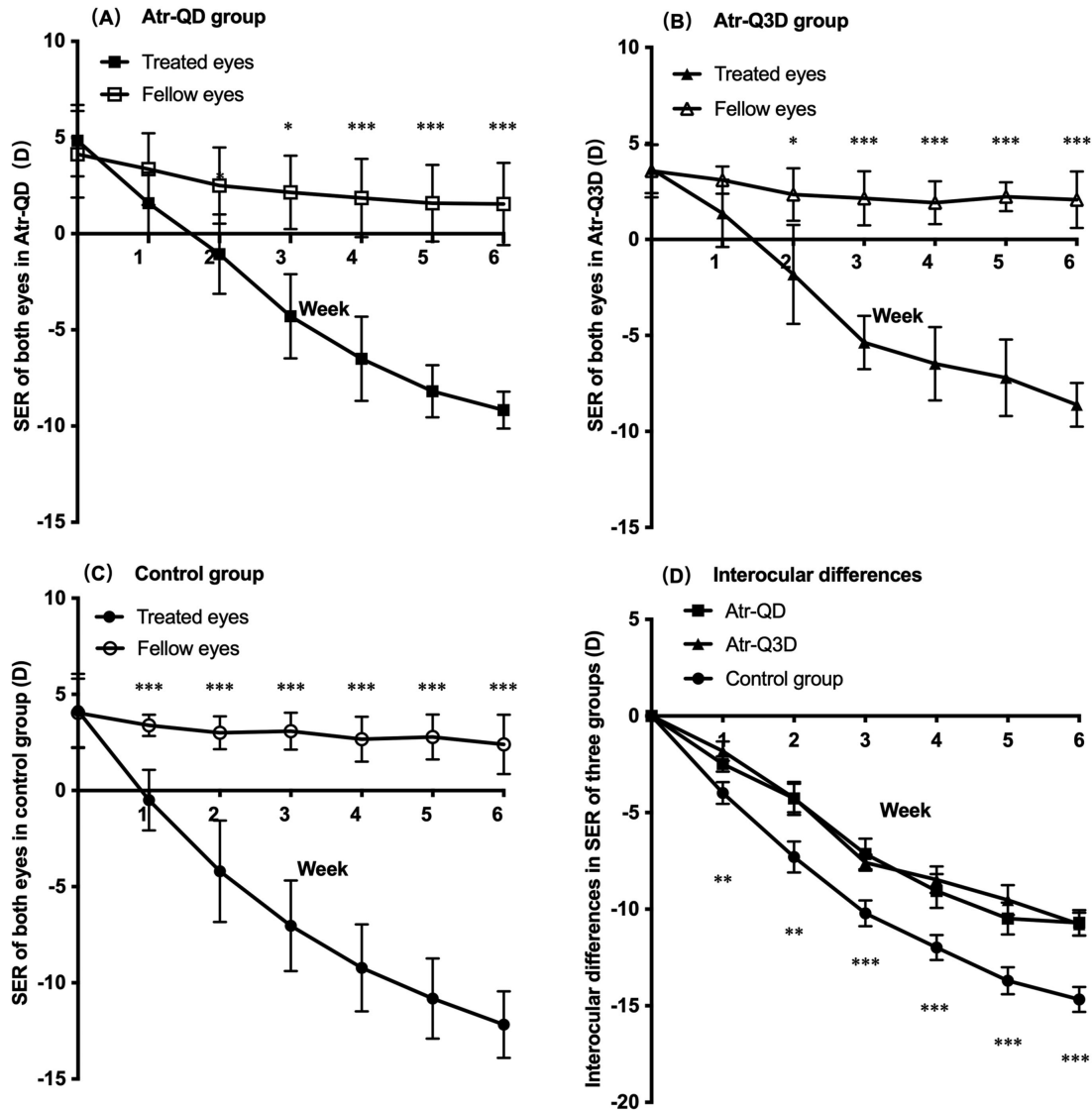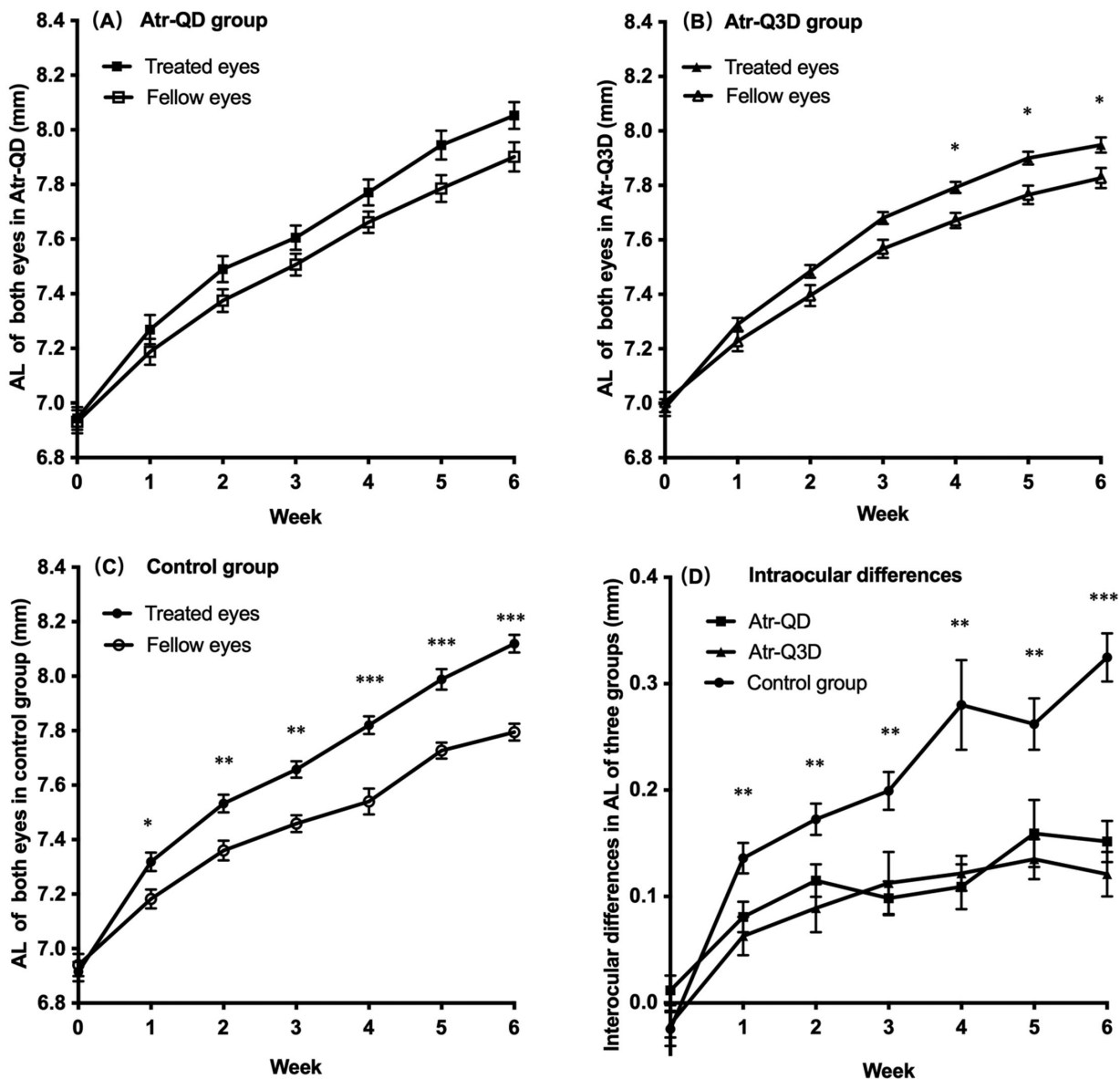
Figure 1. SERs (Mean ± SEM) plotted against time for guinea pigs wearing a negative contact lens on their right eyes for 6 weeks (−10 D, 0–3 weeks, −15 D, 3–6 weeks), with the same eyes treated with topical 1% atropine daily (Atr-QD) (**A**), or every 3 days (Atr-Q3D) (**B**), or with artificial tears daily (control) (**C**); interocular differences in SERs (mean ± SEM), recorded for the same 3 groups and time intervals shown in (**D**). $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

significantly by the end of week 3, which marked the end of the −10 D lens wearing period (0.10 ± 0.22 D vs. -10.12 ± 0.58 D, $P < 0.001$), and a further increase in relative myopia was seen over the second 3-week period during which −15 D lenses were worn. The interocular difference in SER at the final measurement time point was -14.67 ± 0.61 D ($P < 0.001$). These changes in SER contrast sharply with findings for the two atropine-treated groups, which showed significantly smaller treatment-induced changes in SER; interocular differences at the same time points were -6.44 ± 0.71 D and -7.50 ± 0.41 D at week 3 ($P < 0.001$), and -10.71 ± 0.61 D and -10.68 ± 0.55 D at week 6 ($P < 0.001$) for the Atr-QD and Atr-Q3D groups, respectively (Fig. 1).

Axial lengths (ALs): The myopic changes in lens-wearing eyes just described reflect relatively faster increases in their ALs compared to their fellows, and consistent with the intergroup refractive error differences just described, there were also significant differences between the control group and two atropine-treated groups in interocular differences in AL ($P < 0.001$) (Fig. 2). The control group showed the largest increase in AL in lens-wearing eyes. For this group, the interocular difference in AL had reached 0.20 ± 0.02 mm ($P < 0.001$) at the end of week 3, and 0.36 ± 0.03 mm ($P < 0.001$) at the end of week 6, with these changes being significantly greater than those of both atropine-treated groups, which recorded equivalent differences of 0.10 ± 0.02 mm and 0.15 ± 0.02 mm,
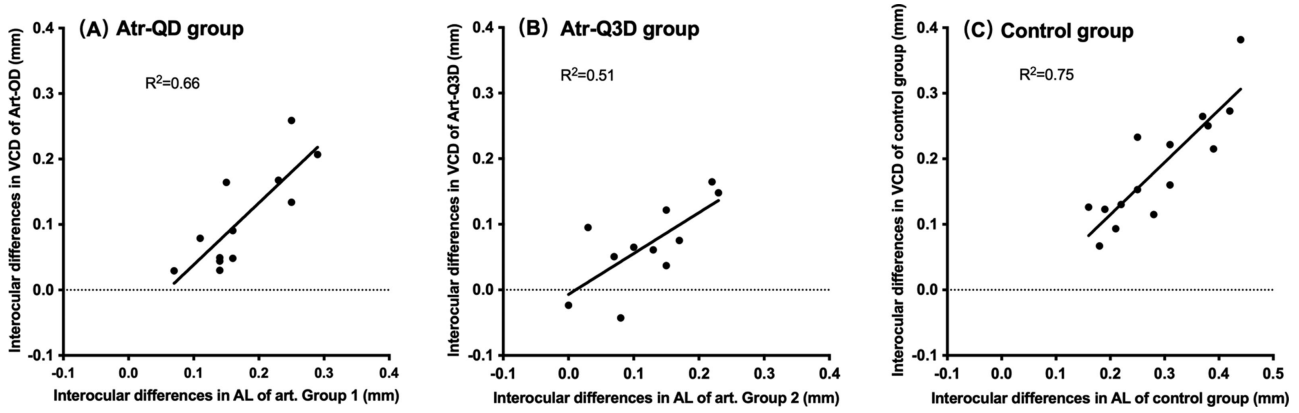
Figure 2. ALs (mean ± SEM) plotted against time for guinea pigs wearing a negative contact lens on their right eyes for 6 weeks (−10 D, 0–3 weeks, −15 D, 3–6 weeks), with the same eyes treated with topical 1% atropine daily (Atr-QD) (**A**), or every 3 days (Atr-Q3D) (**B**), or with artificial tears daily (control) (**C**); interocular differences in ALs (mean ± SEM), recorded for the same 3 group and time intervals shown in (**D**). *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

in the case of the daily atropine (Atr-QD) group and 0.11 ± 0.03 mm and 0.12 ± 0.02 mm in the group receiving atropine every 3 days (Atr-Q3D). The AL changes in the contralateral fellow eyes of the three groups were not significantly different from each other ($P = 0.269$), implying that atropine had no contralateral effect.

Other axial ocular parameters and corneal curvature: To verify that interocular differences in AL reflected increases in VCD, as characteristic of myopia, correlations between the interocular differences in VCD and AL recorded at the end of week 6 were

examined for each group (Fig. 3). For all three groups, these parameters were highly correlated (Atr-QD: $r^2 = 0.66$, $P = 0.001$; Atr-Q3D: $r^2 = 0.50$, $P = 0.015$; control: $r^2 = 0.75$, $P < 0.001$), implying that increased VCD elongation largely accounted for the increases in the ALs of lens-wearing eyes. Interocular differences in VCD at the end of the 6-week treatment period for guinea pigs treated with atropine daily or every 3 days were also significantly smaller (0.10 ± 0.02 and 0.07 ± 0.02 mm, respectively) than that of the control group (0.18 ± 0.02 mm, $P < 0.001$). None of the other measured axial parameters (CCT, ACD,

Figure 3. Plots of interocular differences in VCD against AL at week 6 for Atr-QD group (**A**), Atr-Q3D group (**B**), and control group (**C**); all showed strong correlations.
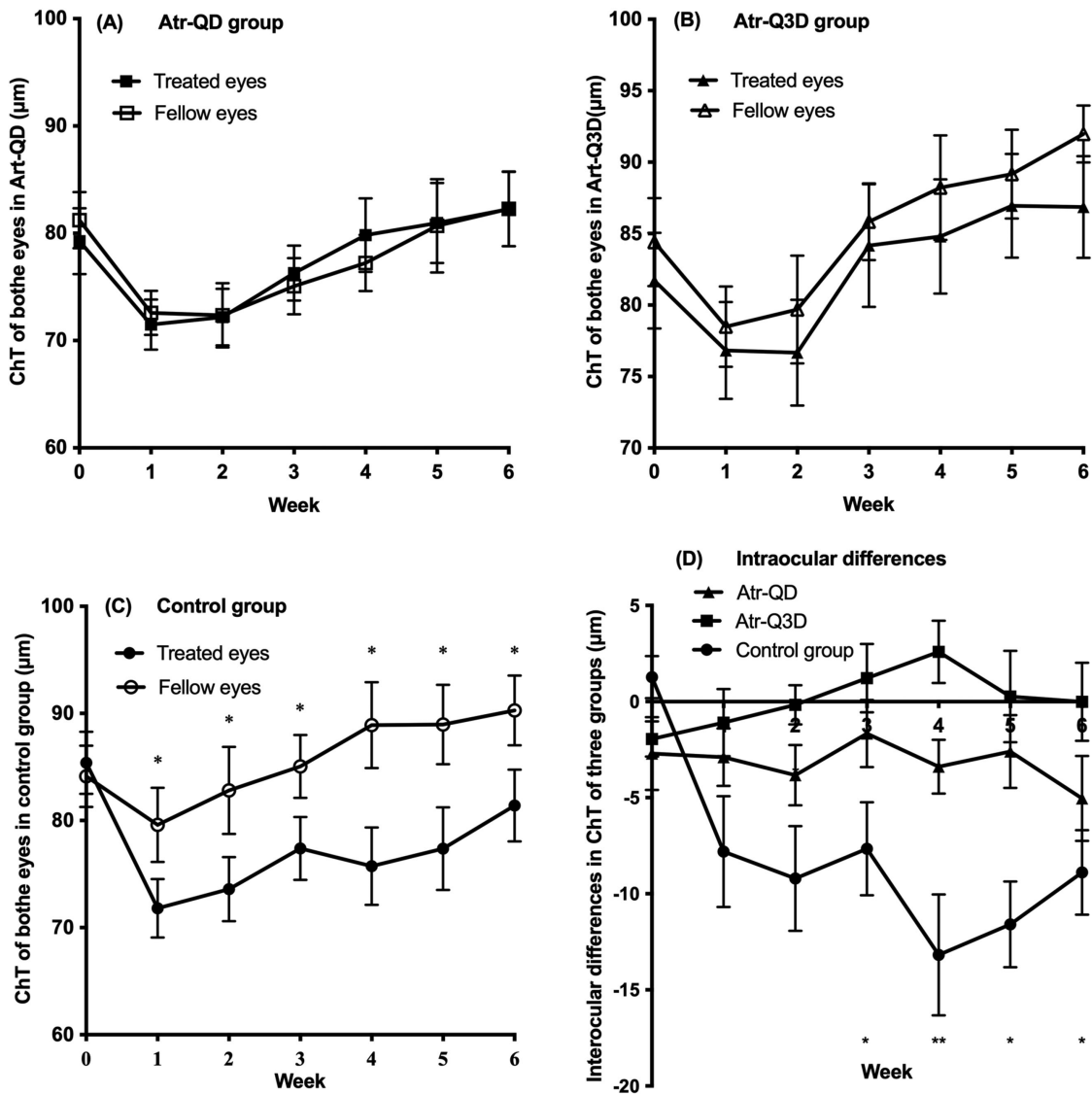
Figure 4. ChTs (mean ± SEM) plotted against time for guinea pigs wearing a negative contact lens on their right eyes for 6 weeks (−10 D, 0–3 weeks, −15 D, 3–6 weeks), with the same eyes treated with topical 1% atropine daily (Atr-QD) (**A**), or every 3 days (Atr-Q3D) (**B**), or with artificial tears daily (control) (**C**); interocular differences in SERs (mean ± SEM), recorded for the same 3 group and time intervals shown in (**D**). *$P < 0.05$; **$P < 0.01$.
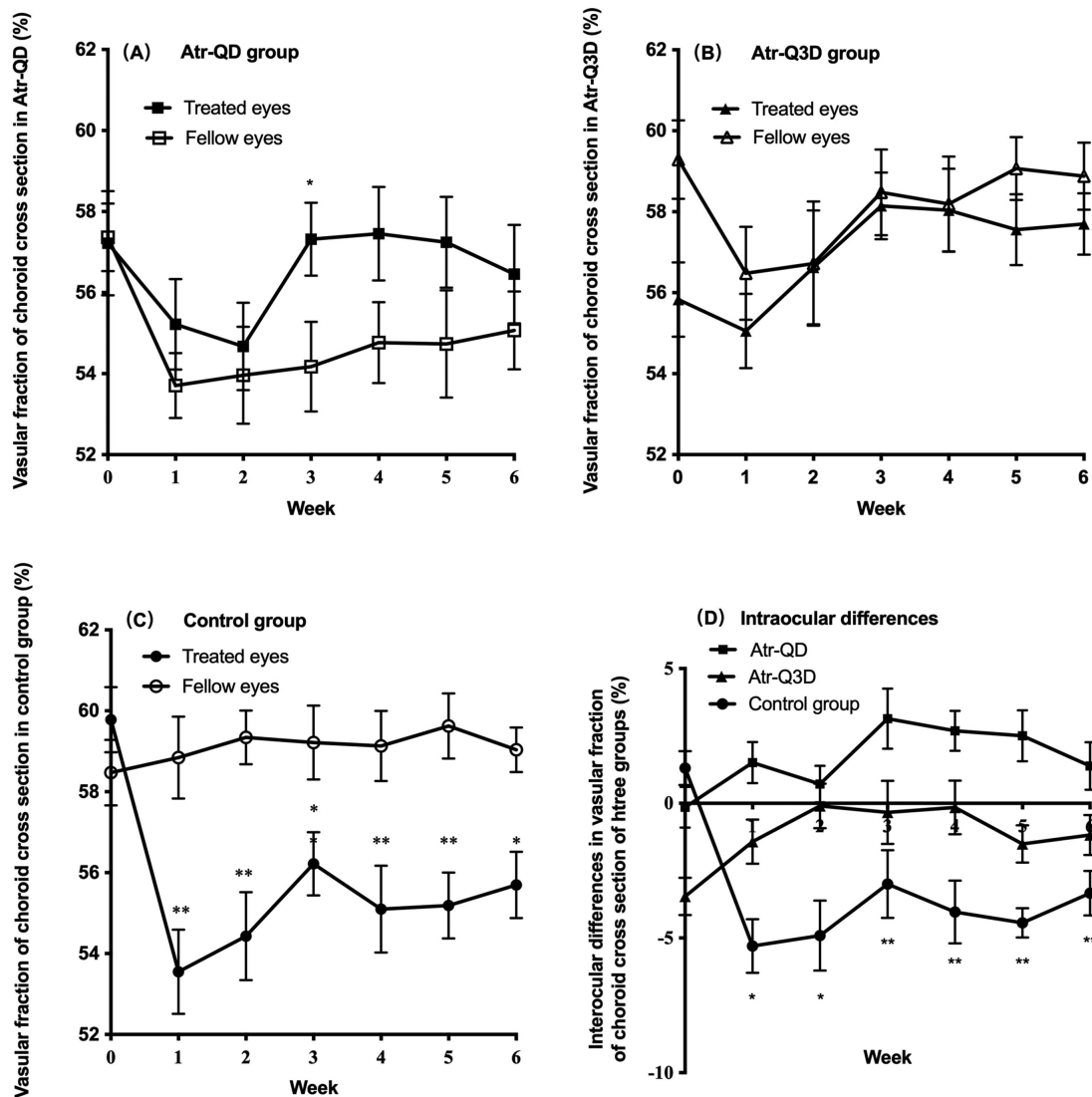
Figure 5. Choroidal luminal area percentage (mean $\pm$ SEM) plotted against time for guinea pigs wearing a negative contact lens on their right eyes for 6 weeks (-10 D, 0–3 weeks, -15 D, 3–6 weeks), with the same eyes treated with topical 1% atropine daily (Atr-QD) (**A**), or every 3 days (Atr-Q3D) (**B**), or with artificial tears daily (control) (**C**); interocular differences in SERs (mean $\pm$ SEM), recorded for the same 3 groups and time intervals shown in (**D**). *$P < 0.05$; **$P < 0.01$.

and axial lens thickness), showed significant changes in interocular differences over time, nor were there significant differences between the groups in terms of changes in interocular differences. Likewise, the central corneal radius of curvature showed no treatment-related changes (Supplementary Fig. S1).

Effects of Myopia Induction on Retinal and Choroidal Thickness and Ameliorating Effects of Atropine

Retinal thickness (RT): Lens-wearing eyes and their fellows showed similar decreases in RT over the treat-

ment period. Thus, for each group, interocular differences in RT at the end of the experiment were close to that recorded at baseline and there were also no significant differences in interocular differences in RT between the groups (Supplementary Fig. S2).

Choroidal thickness (ChT): In contrast to the lack of treatment effects on RT, the myopia induced in the lens-wearing eyes of the control group was linked to decreases in ChT, whereas the fellow eyes of the group recorded slight increases over the same period. The changes over time in interocular differences in ChT for this group reflect these contrasting patterns of change, becoming significantly more negative, from 1.27 ± 1.10 μm at week-0 to -7.66 ± 2.42 μm by the end of

week 3 ($P = 0.002$) and -8.89 ± 2.21 μm at the end of week 6 ($P = 0.002$). On the other hand, the two atropine groups recorded only small interocular differences in ChT that were relatively stable over the treatment period (1.22 ± 1.78 and 1.67 ± 1.85 μm, week 3; -0.01 ± 2.03 and -5.10 ± 2.33 μm, week 6, for Atr-QD and Atr-Q3D groups, respectively). The interocular differences in ChT for the two atropine groups were also both significantly different from those of the control group at week 3 and week 6 ($P = 0.015$, and $P = 0.021$; Fig. 4).

Myopia-related Structural Changes in Choroid and Effects of Atropine

The ChT data just described imply that atropine, either directly or indirectly inhibited myopia-related choroidal thinning. The structural analysis of captured SD-OCT choroidal images aimed to better understand this effect. For the lens-wearing eyes of the control group, the ratio of blood vessel area to total choroidal area gradually decreased over time, while that of their fellows remained almost unchanged. By comparison, the equivalent ratios derived for both the lens-wearing and fellow eyes of the two atropine groups remained relatively stable over the course of the experiment. This difference between the control and atropine groups is also reflected in the interocular differences in this ratio, which changed significantly for the control group (from $1.30 \pm 0.63\%$ at baseline to $-3.34 \pm 0.83\%$ at week-6, $P = 0.006$), with the final interocular difference also being significantly different from those of the two atropine groups (Atr-QD group: $1.38 \pm 0.88\%$ at week 6, Atr-Q3D group: $-1.18 \pm 0.74\%$ at week-6, $P < 0.001$; Fig. 5).

Effects of Atropine on Pupil Responses

In adult guinea pigs, one drop of topical 1% atropine resulted in pupil dilation within 1 hour of instillation and pupils also became unresponsive to light stimulation, with these effects lasting up to 4 days. Similar effects were observed in eyes treated daily with topical 1% atropine, with no apparent loss of these treatment effects over the 5-week monitoring period.

Discussion

This study explored whether topical 1% atropine is effective in slowing or arresting contact lens-induced myopia progression in pigmented guinea pigs, and, importantly, whether daily administration of atropine was critical to such treatment effects. We found that instillation of topical 1% atropine, either daily or every 3 days were equally effective in slowing myopia progression.

Customized negative RGP lenses were used to induce myopia in this guinea pig study. Although the more commonly used approach to myopia induction in guinea pigs involves spectacle lenses, they can become detached as the animals move around in their cages, interrupting the myopia-inducing visual experience, in this case hyperopic defocus. The contact lenses used in the current study largely avoided this problem, being rarely dislodged and so providing a continuous defocus experience. In the study reported here, contact lens-wearing eyes also received on a daily basis either topical 1% atropine or artificial tears and while the atropine solution was preserved with benzalkonium chloride, we observed no adverse corneal effects over the study period despite the well-documented cytotoxicity of this preservative.

The left eyes of all animals were left untreated, thereby serving as contralateral controls. Over the 6-week monitoring period, these eyes showed a gradual reduction in their hyperopic refractive errors, flattening of their corneas, and increases in all axial dimensions, with the exception of retinal thickness, which decreased. These changes are consistent with normal ocular development and emmetropization, as reported by others,[26,27] also implying minimal treatment-related interocular yoking.

This study represents the first to demonstrate the efficacy of topical atropine sulfate solution in the guinea pig defocus model of myopia, whereas the finding that atropine inhibits myopia progression is not in of itself new. The only other published study into the efficacy of atropine in controlling myopia in guinea pigs involving form deprived myopia (FDM) animals, which received a daily peribulbar injection of atropine sulfate monohydrate over a 2-week period.[28] This atropine treatment regimen was found to reduce the amount of induced myopia and related increase in axial length by approximately 61% and 58%, respectively. Although the latter changes are much larger in percentage terms than those reported here (i.e. 27% and 58% for Atr-QD, and 25% and 66% for Atr-Q3D), there are potentially important differences in study design parameters, including the age of the animals (3 vs. 2 weeks at baseline), treatment durations (2 vs. 6 weeks), and route of administration (peribulbar injection vs. topical). That two different myopia inductions method were used (form deprivation versus hyperopic defocus), may also be significant, given the evidence that different underlying retinal mechanisms are involved.[29] In addition, the form deprivation model is considered "open loop," in that the induced ocular growth changes

have minimal effect on the imposed retinal image degradation driving the changes. On the other hand, the same growth changes serve to progressively compensate for imposed hyperopic defocus. We incremented the power of the contact lenses mid-way through the treatment period in our study in an attempt to minimize the decrease in imposed defocus. However, it is important to note that the inhibitory effects on axial elongation are similar across our two studies, reflecting the complex interactions between the contact lens and "tear lens" beneath the former, which together determined the imposed defocus. The power of the latter is also expected to change over time, due to developmental corneal flattening. Finally, although no significant contact lens-induced changes in corneal curvature were recorded, it is possible that subtle changes in the latter may also partly account for the apparent mismatch in inhibitory effects on myopia progression versus axial elongation.

Of relevance to the study reported here, a recent clinical study found no significant differences in SER changes over 15 months in the effects of 1% atropine administered once (0.26 ± 0.70 D), twice (0.51 ± 0.70 D), and three times per week (0.46 ± 0.76 D).[30] In the current study, topical atropine instilled every 3 days proved to be as effective as daily treatment in inhibiting lens-induced myopia in our young pigmented guinea pig subjects. These results suggest an enduring action of topical atropine and are consistent with observed sustained pupil dilation with topical atropine in our adult guinea pigs (see Supplementary Fig. S3). For example, the mydriatic effect of a single drop of 1% topical atropine in these animals lasted 3 to 4 days. Although the ocular site of action for the anti-myopia effect of atropine remains under debate, it is reasonable to assume a similar or longer duration of action in young guinea pigs, given the smaller size of their eyes and hence likely higher intraocular concentrations. With repeated dosing, it is also likely that atropine accumulated over time in pigmented ocular tissues (iris, ciliary body choroid, and retinal pigment epithelium), bound to melanin, thereby creating local intraocular depots. Slow release from such depots allows for sustained receptor blockade.[31] Depending on the ocular site of action of atropine's anti-myopia effect, a longer dosing interval might thus also be plausible.

In relation to the above speculation, parallels may be drawn between reports from human clinical and animal studies. For example, in one study, delayed but longer lasting cycloplegia and mydriasis have been reported in human individuals with more heavily pigmented eyes.[32] That the mydriatic effects of topical atropine outlast its cycloplegic effects, by 1 to 2 days as reported in another human study, may reflect the relative tissue

levels of melanin, although the iris is likely to be also exposed to a higher concentration of atropine, based on pharmacokinetic principles.[14] Moreover, a study in mature rabbits, whose eyes are more similar in size to those of humans (17–18 mm equatorial versus 16 mm axial dimensions)[33,34] found detectable levels of atropine in various ocular tissues, including ciliary body, retina, and sclera, 3 days after instillation of 1% atropine.[35] Taken together, these findings argue for investigation into the efficacy of additional less frequent dosing schedules, and also to examine the interacting effects of dosing concentration and interval.

Although as already indicated, there is no general agreement on the ocular site of action of atropine's anti-myopia action, there is accumulating evidence that it can prevent, directly or indirectly, the choroidal thinning that typifies myopic eyes. The choroid lies between the retinal pigment epithelium and the sclera, and is the main source of nutrients to the outer retina and sclera.[36] That the choroid may also play an important role in early eye growth regulation and emmetropization is supported by both clinical and animal model studies. Specifically, choroidal thinning and reductions in blood flow have been reported in myopic human eyes, as well as animal eyes with experimentally induced myopia, with the opposite effects seen under conditions that slow eye elongation and so slow or reverse myopic changes. For example, exposure to myopic defocus, either imposed optically with positive lenses, or after myopia-inducing treatments are terminated, has been linked to increased blood flow and choroidal thickening, at least in animal model studies.[37–43] In our study, the contact lens-wearing eyes of the control group showed relative choroidal thinning compared to their fellows, by approximately 11% and the lumens of choroidal blood vessels had decreased by approximately 8% by week 6. In contrast, with topical atropine, instilled either daily or every 3 days, the choroids showed very slight increases in thickness relative to their fellows (1–2%) and choroidal blood vessel area was almost unchanged in both groups. Our findings also have a parallel in the findings from the FDM guinea pig study referred to above, which reported slight choroidal thickening in FDM eyes with peribulbar injection of atropine, by approximately 6 μm relative to those injected with saline; choroidal perfusion was also relatively higher with peribulbar atropine-compared to saline-injections.[28] Overall, it appears that atropine applied locally on or near the eye can protect against choroidal thinning in eyes exposed to myopia-inducing stimuli.[28,39,44]

The mechanism underlying the effects of atropine on choroidal thickness and blood vessel area remains

unclear. In the current study, the observed decrease in choroidal blood vessel ratio in the lens-wearing eyes in the control group largely reflected decreases in blood vessel areas (from $27{,}380.19 \pm 1362.06$ μm^2 at baseline, to $25{,}808.77 \pm 1411.22$ μm^2 at week 6), whereas the stromal area remained almost unchanged ($18{,}152 \pm 538.22$ μm^2 at baseline and $18{,}471.22 \pm 847.89$ μm^2 at week 6). Of potential relevance to the inhibitory effect of atropine on the above changes is a study in chickens, which linked the inhibitory effect of atropine on form deprivation myopia to increased nitric oxide (NO) synthesis.[45] Inhibition of NO synthesis was also found to inhibit the choroidal thickening response to imposed myopic defocus in chicks.[46] As a plausible explanation for the inhibitory effect of atropine, the involvement of NO as a mediator is also consistent with the well-documented vasodilatory action of NO, although nitric oxide synthase (NOS)-positive axon terminals have also been colocalized with choroidal nonvascular smooth muscle cells, consistent with an alternative, nonvascular mechanism for NO-mediated effects on choroidal thickness.[36] These and other alternative mechanisms for the observed choroidal effects of atropine warrant further investigation.

In the current study, the fellow, untreated eyes of all three groups appeared unaffected by the applied treatments, be it imposed hyperopic defocus combined with artificial tears, or hyperopic defocus combined with topical atropine. In the case that atropine was able to reach the fellow eyes intact, this result would indicate that atropine does not interfere with normal emmetropization, a potentially important observation with respect to atropine's clinical use for myopia control in that it allows for its use prior to the onset of myopia in high-risk children. While the distribution and metabolism of atropine in our guinea pig model awaits further study, one study on rabbits using monocular topical 1% atropine reported crossing-over effects on the untreated eye within 5 hours post-administration.[35] Perhaps of greater relevance, a study involving monocular intravitreal injection of atropine and binocularly form deprived chicks reported retarded myopia progression not only in the atropine-injected eye but also in the saline-injected contralateral eye.[47] Nonetheless, in a further study involving monocularly form deprived chicks, monocular intravitreal injection of atropine did not slow down emmetropization in the contralateral eyes.[48]

As alluded to above, previous studies investigating the anti-myopia effects of atropine in animal models have used a variety of routes of administration, with ocular pharmacokinetics expected to vary, depending on the choice. Both intravitreal and peribulbar injection have been widely used in animal model studies.[28,49,50] These routes of delivery offer the advantage of good control of the volume and thus the local concentration of the applied drug, for example, in dosing studies. As examples, intravitreal injections of 2.5 μg and 250 μg atropine were found to inhibit form-deprivation myopia in chickens by 50% and 100%, respectively, with injections of slightly higher doses (i.e. 25 μg and 750 μg atropine yielding similar inhibitory effects [50% and 100%]) on negative lens-induced myopia.[47] However, because of their relatively invasive nature, regular injections are not suitable for clinical adoption, especially in children. On the other hand, the results from the current study, which used topical delivery, are potentially translatable to clinical practice.[51,52]

Conclusion

In summary, our study found that administration of 1% topical atropine, either daily or every 3 days, similarly slowed the progression of defocus-induced myopia (lens-induced myopia [LIM]) in guinea pigs, opening up the possibility that instillation frequency can be reduced from the widely used daily dosing regimen without loss of efficacy for myopia control in children. Furthermore, we found that 1% topical atropine inhibited myopia-related choroidal changes, as observed in LIM eyes treated with artificial tears. Although the mechanism(s) underlying atropine's myopia control effect remains to be fully resolved, our results add to accumulating evidence pointing to involvement of the choroid.

Acknowledgments

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