Effects of Monocular Atropinization on Refractive Error and Eye Growth in Infant New World Monkeys

Andrew R. Whatham,* Daniel Lunn,† and Stuart J. Judge

Correspondence: Stuart J. Judge, Department of Physiology, Anatomy & Genetics, Sherrington Building, Parks Road, Oxford OX1 3PT, UK; stuart.judge@dpag.ox.ac.uk.

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Purpose. To explore the effect of topical atropine on axial eye growth and emmetropization in infant marmosets.

Methods. Atropine was applied to one eye from the age of 7 to 56 days in two dose regimens, High (0.1–1% twice daily, increasing with age) or moderate (Mod) (0.1% once daily). Both eyes of the marmosets were refracted, and axial dimensions were measured ultrasonically, at 14, 28, 42, 49, 56, 70, 105, 168, and 279 days of age. The time course of each measured variable was analyzed using multilevel mixed-effects modeling realized in R.

Results. The logistic growth curves fitted to anterior segment depth (ASD) did not differ significantly between the dose regimens, but xmid, the age at which growth was maximal, and scal, the time constant of the exponential term in the logistic growth curve equation, differed significantly between the ASD of atropinized and untreated eyes (P = 0.03 and P < 0.0001, respectively), with the ASD of atropinized eyes shorter than that of untreated eyes. The splines fitted to lens thickness did not vary significantly with dose, but differed significantly (P < 0.0001) between the atropinized and untreated eyes, with the atropinized lenses thicker. Vitreous chamber depth (VCD) was not significantly different, but the variance of VCD was significantly greater (P < 0.001) in the atropinized compared with the untreated eyes. Refractive error (RE) became relatively myopic in atropinized eyes. The variance of RE in atropinized eyes was significantly greater (P < 0.0001) than in untreated eyes.

Conclusions. Atropine caused the infant marmoset lens to move forward and thicken, a relative myopia, and increases in the between-animals variance in VCD, which could be considered a failure of emmetropization.

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Atropine Administration
Atropine sulphate solution (1% minims obtained from Chauvin Pharmaceuticals Ltd, Romford, UK) was administered once or twice daily to one eye of 10 infant marmosets from 1 week of age until 8 weeks of age. Howlett and McFadden\(^27\) suggest that at the age of 8 weeks the ocular development of marmosets is approximately equivalent to that of humans at the age of 18 months. Marmosets were treated with a maximal dose of atropine (High group), which was defined as the largest dose that avoided a systemic effect, which was defined as any degree of dilation of the contralateral pupil. These marmosets received two drops of atropine per day, the first just after the onset of the light period and the second 6 hours later, halfway through the 12-hour light period. The concentrations of atropine administered to these marmosets were 0.1% from 1 to 4 weeks of age, 0.3% from 4 to 6 weeks of age, and 1.0% from 6 to 8 weeks of age. The remaining five marmosets received one drop of 0.1% atropine solution each day (Mod group) 1 hour after the onset of the light period. All atropine solutions less than 1.0% concentration were obtained by diluting the 1.0% atropine sulphate solution with sterile physiological saline. The total volume of a single eye drop was estimated to be 25 \(\mu\)L. The eye drops were allowed to sit on the eye for a short period of time (up to 1 minute) before the area surrounding the eye was dried using a cotton bud to prevent contamination of the fellow eye with atropine. Occasionally the pupil of the contralateral eye was partially dilated for a short period following atropine administration. If this did occur, the administration duration for the next drop (the following day) was decreased to reduce the possibility of systemic levels of atropine interfering with eye growth. In this way the atropine dose could be adjusted by increasing or decreasing the ocular surface contact time for the drug for each marmoset to give the maximum cycloplegic effect and the minimum systemic dose, for the concentration of atropine used. Throughout the period of atropine treatment all the pupils of the atropinized eyes of the High group and four of the five eyes in the Mod group were dilated and unresponsive to light. On one occasion, the pupil of the atropinized eye in one of the marmosets in the Mod group was maximally dilated 1 hour after the administration of atropine but was approximately two-thirds dilated at the next administration time the following day.

Optometric Measurements
Under cycloplegia (one drop of 1.0% cyclopentolate), refractive error (RE) was determined retinoscopically with the animals unanesthetized. Axial ocular dimensions were measured ultrasonically (Ophthalmasonic A scan III; Mentor Medical, Lancing, UK), with a 7.28-MHz probe capped by a 15-mm stand-off. Resolution of the anterior and posterior surfaces of the cornea was not always possible, so we measured the sum of the corneal thickness and anterior chamber depth, which we refer to as anterior segment depth (ASD).\(^28\) For these measurements the animals were anesthetized with an intramuscular injection of 0.9% alphaxalone/0.3% alphadolone acetate (Saffan; Schering-Plough Animal Health, Welwyn Garden City, UK). At the measurement points during atropine administration (14, 28, 42, 49, and 56 days of age), no cyclopentolate drops were given to the eye receiving atropine in order to avoid the possibility of cycloplegia interfering with the level of cycloplegia provided by the atropine dose. The first measurement of refraction and ocular dimensions was at 14 days of age (7 days after the commencement of atropine administration), with the exception of two marmosets from the maximum-dose atropine group in which the first measurement point was at 28 days of age (3 weeks after the commencement of atropine treatment). Marmosets were not measured at 1 week of age to avoid separating them from their mothers within 2 weeks from birth for 3 hours: the period of time required for cycloplegia, refraction, anesthesia for ultrasound measurements, and recovery from anesthesia.

Measurement of Amplitude of Accommodation
The amplitude of accommodation of one marmoset from each treatment group was measured at approximately 4 or 5, 7, and 8 weeks of age (during the period of atropine administration) using dynamic retinoscopy (retinoscopy performed without additional cycloplegia while the marmosets were accommodating). At each measurement point the amplitude of accommodation of the atropinized eye was measured before the atropine dose, 1 to 1.5 hours after the dose, and 6 hours after the dose in the High group, and 9 to 9.5 hours after the dose in the Mod group. The marmosets were placed in a marmoset stand\(^29\) while awake and fully alert. A small object of interest to the marmoset, such as a piece of dried banana or...
sunflower seed, was used to attract the attention of the animal. This object of interest was held approximately 10 to 15 cm in front of the marmoset at a height such that the marmoset could view the object in primary gaze. Streak retinoscopy was then performed, in the vertical meridian only, while the marmoset was looking as closely as possible along a direct line of sight to the retinoscope. The retinoscopist maintained a constant working distance of 50 cm (2 D) from the corneal plane. Increasingly negative-powered trial lenses were positioned 15 mm in front of the animal, between the marmoset and the object of interest, until no "against movement" of the retinoscopic reflex was observed when the marmoset viewed the object through the lens. The power of the most negative or least positive-powered trial spectacle lens through which "against movement" was observed was taken to represent the limit of the animal's accommodative capacity. The amplitude of accommodation was calculated to be the difference between the nominal RE without correction for the artifact of retinoscopy, measured by retinoscopy in the anesthetized marmoset, and the most negative or least positive-powered trial spectacle lens through which "against movement" of the retinoscope beam was observed in the awake marmoset. In all calculations the retinoscopy measurement in the vertical meridian was used.

**Statistical Analysis**

Untreated and atropinized eyes belonged to the same animal, so the measurements comprising the data were paired. Therefore multilevel modeling was required with animal ID as a level. R version 3.5.1 (R Foundation, Vienna, Austria) was used with the libraries nlme and splines to fit statistical models to account for the change with age of the measured variables (ASD, lens thickness, RE, and vitreous chamber depth).
with eye (atropinized or untreated) and dose included as interactions. The built-in variance functions from the nlme library were incorporated into the models to allow for and test different treatment regimens having different residual variances. Linear regression was carried out with Type II linear models by using the lmodel2 library in R, because there were measurement errors in both variables.

The fixed-effects residuals and the random-effects residuals were checked for normality and homoscedasticity for all of the fitted models and all were satisfactory. Plots of measured values against fitted values were also made and showed excellent fit.

RESULTS

Anterior Segment Depth

The data on ASD are shown in Figure 1. They were fitted with a logistic growth curve

\[ \text{asd} = \frac{\text{Asym}}{1 + \exp \left( \frac{\text{age} - \text{xmid}}{\text{scal}} \right)} \]

using the nlme() function in R after symmetrizing the data distribution along the time axis using the transform \( tage = \text{age}^{0.1} \). The parameter Asym is the final (asymptotic) value of anterior segment depth (asd); xmid is the age at which growth is half-maximal, and scal is a time constant, variation of which affects both the initial value and the growth rate.

Random effects were needed for both the asymptote (Asym) and the point of inflection of the growth curve (xmid) to account for the data. Dose was not significant. The growth curves in the two eyes were significantly different (\( P = 0.03 \) and \( P < 0.0001 \) for xmid and scal, respectively) but the asymptotes in the untreated and atropinized eyes were not significantly different (\( P = 0.11 \)). See Figure 2.

Lens Thickness

The lens thickness data are shown in Figure 3. They were fitted with a cubic spline model. Dose was not significant. Fitting the data from the untreated and atropinized eyes with the same cubic spline made for a significantly poorer (\( P < 0.0001 \)) fit.
than fitting the two eyes with separate splines. See Figure 4 for the fitted curves.

**Refractive Errors**

Figure 5 shows REs of the untreated and atropinized eyes. These data were fitted with cubic splines, separately for the two doses (Fig. 6). The atropinized eyes became approximately 1.5 D relatively myopic compared with the untreated eyes (Fig. 6). Both eye and dose had significant effects ($P = 0.001$ and 0.0015, respectively). Variances in the atropinized eyes were highly significantly greater ($P < 0.0001$) than those in the untreated eyes. Note that by the age of 56 days at which atropinization ceased, both groups of animals had grown out of the variable hyperopia characteristic of the first few weeks of life.28

**Vitreous Chamber Depth**

Over the period of life studied, marmoset VCD varies linearly with log(age). We therefore fitted the vitreous chamber data with a (natural) logarithmic function, together with fixed effects for dose and eye. Neither dose of atropine nor whether atropine was given had a significant effect ($P = 0.23$). As with RE, the variance of VCD was highly significantly greater ($P < 0.001$) than in the untreated eyes (Fig. 7).

**Failure of Emmetropization During the Period of Atropinization**

Early in life, animals vary in RE and VCD, but they grow so as to reduce these interanimal differences and become emmetropic, or nearly so. Figure 8 shows that if one plots the change in refraction and VCD between the ages of 14 and 56 days as a function of initial refraction at the age of 14 days, then, whereas in the untreated eyes (filled circles) refraction decreases (95% confidence interval [CI] on slope $0.657$ and $1.699$) and VCD increases (95% CI on slope $0.033$ and $0.102$) with initial refraction, in the atropinized eyes (crosses) there was no such correlation (95% CI on slope $-0.919$ and $0.778$ and $-0.66$ and 0.02) for refraction and VCD changes with initial refraction, respectively.

The disruption of emmetropization associated with atropinization was largely reversible in that after the cessation of atropine, the difference in RE between treated and untreated eyes disappeared. See Figures 5 and 6.

**Accommodation**

Throughout the period of atropinization, the amplitude of accommodation of the untreated fellow eyes of the two marmosets was greater than 30 D.

The mean accommodative amplitude for the atropinized eye in the High group was 6.5 and 11 D at 34 and 48 days of age, respectively (i.e., after 26 and 41 days of atropine treatment). The range of measures throughout the day at each of the three ages for this animal was not greater than 2 D.

In contrast, the amplitude of accommodation in the atropinized eye of the infant in the Mod group varied considerably during the day. At 26 days of age the amplitude of accommodation of the atropinized eye was 6 D 1 hour after and 10 D 9 hours after the atropine dose. At 44 days of age the amplitude had increased to 10 D 1 hour after and 31 D (i.e., approximately normal) 9 hours after the atropine dose.

**DISCUSSION**

**Effect of Atropine on Refractive Error and Vitreous Chamber Depth**

The effect of atropine on RE was not permanent, with the difference in relative error between atropinized and control eyes disappearing by the last measurement. Refractive error was on average more myopic in atropinized eyes than in untreated eyes, and much more variable between animals. On the other hand, the effect of atropine on VCD was only to increase the variance of VCD. We interpret these effects on RE and vitreous chamber as a disruption of emmetropization.31 One obvious point is that the relative myopia was not associated with increased VCD. It is possible that the relative
myopia was caused by the decrease in ASD (less the opposing changes in lens thickness), but we cannot exclude other possibilities such as an effect of atropine on corneal curvature. In the rabbit, topical atropine reaches the vitreous and also retina, so it is possible that some of the effects of atropine we observed arose from scleral or retinal effects. It is possible that the atropinized eyes became amblyopic. In the macaque, amblyopia is associated with the development of hyperopia, and eyes that develop amblyopia do not show compensatory changes in ocular growth and RE to defocus imposed by spectacle lenses. In the marmoset we have shown previously that although the same procedures that induced amblyopia in the macaque do not induce hyperopia, they do make eye growth and refractive development erratic as in the current studies.

We should stress that in these experiments atropine was applied in animals whose eyes were still emmetropizing, and not at later stages of growth that might be developmentally comparable to the myopic children in grade school whose eyes are treated with atropine. In humans, anterior segment development is rapid in infancy but has largely stabilized by 18 months of age with close to stability of corneal curvature by then, many years prior to the age at which myopia usually starts and atropine treatment is used.

Anterior Effects

The most lasting effects in our findings are the reductions in depth of the anterior segment and the smaller increases in lens thickness. At the last measurement point (273 days old) anterior segments were 0.08 and 0.11 mm shorter in the High and Mod animals, while the lenses were 0.04 and 0.08 mm thicker, respectively. Using the schematic eye described in Troilo et al., we calculated that these changes (without other changes) would alter refraction by $-0.50$ and $-0.68$ D, and $-0.16$ and $-0.32$ D, respectively—relatively small changes compared with those occurring as the eyes grow. Human studies of atropinization find either small effects in the opposite direction—thinner lenses and deeper anterior chamber depths—or no significant effects, though it should be noted that the latter was a between-groups comparison rather than (as in our studies) a between-eyes comparison and so would be expected to have a lower sensitivity.

Comparing Effects of Doses of Topical Atropine in Eyes of Different Sizes

We will first consider the issue of comparing the effects of topical doses of atropine between species of different eye size. During the period of atropinization, marmoset eyes had axial lengths between 6 and 7 mm. This is between a third and a quarter of that of juvenile human eyes. If we assume that absorption is proportional to the area of the conjunctiva, and effect inversely proportional to the volume of the eye, then doses would be three or four times greater in marmosets than in humans. This would make our moderate doses equivalent to one drop of 0.3% or 0.4% atropine in humans—between the two established doses of 0.1% and 1%.

Retinal Focus

One of the problems of experiments on myopia is that it is difficult to know the magnitude of the focus errors experienced by each eye of the animal. We were not able to measure accommodation during the course of these experiments, but the separate studies in two animals showed that chronic topical atropine did not abolish accommodation, and that during the course of the period of atropinization there will have been some accommodative ability in the treated eyes. This raises the possibility that the animals may have been able to focus intermittently on more distant targets with their atropinized eyes. As limited periods of clear vision protect against deprivation myopia in marmosets, if the animals did focus intermittently with the atropinized eyes, that alone would prevent deprivation myopia developing in those eyes, regardless of whether the doses of atropine we used were or were not adequate to prevent myopia.
Relevance to Human Studies

These experiments are not directly comparable with studies of the effectiveness of atropine in preventing or reducing myopia progression in juvenile humans. Atropine was applied earlier in life in our studies, without refractive correction, and in subjects that were hyperopic rather than myopic or at risk of becoming myopic.

We have shown that early in life, doses of atropine in the same range as those used in human treatment of myopia have effects on the growth of the anterior eye. This suggests that one may want to be cautious about using atropine in human infants during early emmetropization. It is worth noting that 1% atropine is also used clinically for protracted periods to treat amblyopia as an alternative to patching. Strabismic and anisometropic amblyopia generally develops at a younger age than myopia. Amblyopia treatment associated with unilateral congenital cataracts occurs at even younger ages that are comparable to the developmental stage of the animals in this study. Careful monitoring of anterior segment development should be considered in clinical application of topical atropine for prolonged periods in human infants and young children.

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