

Time and Degree of Saturation and Recovery from Chronic 0.1% and 0.05% Atropine Treatment
in the Guinea Pig Model

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ABSTRACT

Background: Atropine eye drops are the most effective treatment to slow the progression of myopia in children. Currently, topical atropine is commonly prescribed at 0.01% concentration for daily use. However, we have not fully explored other possible atropine dosing regimens, and the relationships between atropine concentration, accumulation in the eye, and efficacy in controlling myopia remain unclear. The aim of this study was to evaluate the effect of concentration on trends in atropine accumulation and decline during and following a period of daily atropine dosing in guinea pigs, a popular animal model for myopia.

Methods: Four guinea pigs were randomized to one of two atropine regimen sequences: topical 0.1% atropine followed by topical 0.05% atropine, or topical 0.05% atropine followed by topical 0.1% atropine. Each atropine regimen consisted of a two-week period of daily atropine dosing followed by a two-week recovery period. To track active atropine levels in the eye, pupil diameter and pupil response to light were measured every 2-4 days and topical pilocarpine-induced change in refractive error was measured weekly.

Results: Within-subject comparison showed that concentration did not influence the time to muscarinic receptor saturation by atropine according to any of the three outcome measures ($p > 0.5$). Interestingly, guinea pigs appeared to take longer to recover from 0.05% atropine treatment than 0.1% atropine treatment, although this difference did not reach statistical significance ($p >$

0.15). With the higher (0.1%) concentration, guinea pigs achieved a greater degree of receptor saturation, as indicated by greater reductions in pupil response ($p = 0.034$).

Conclusion: In the guinea pig model, the concentration of topical atropine instilled appears to influence the degree of muscarinic receptor saturation achieved, but not the time to saturation or recovery. These results support the use of pupil response as a biomarker for active atropine levels in the eye.

INTRODUCTION

Myopia as a threat to public health

Myopia, or nearsightedness, is an extremely common vision condition which has seen unprecedented increases in prevalence over the last 50 years.¹ Myopia currently affects about half of college-aged individuals in the United States¹, Europe¹, and Asia² and is projected to afflict nearly half the entire global population by 2050³. In certain myopia “hotspots” in East Asia, nearly 100% of young adults are affected. For example, two recent surveys of military conscripts in Tainan, Taiwan⁴ and Seoul, South Korea⁵ found prevalence figures of 86.1% among 18 to 24-year-old males and 96.5% among 19-year-old males, respectively.

In most cases, myopia can be “corrected” by glasses, contact lenses, or laser surgery. Such corrections resolve myopes’ problem of blurry distance vision, allowing them to see clearly. However, the structural changes associated with myopia—lengthening of the eye and thinning of the eye wall—worsen with higher myopia and increase the risks of retinal detachment, glaucoma, and other potentially blinding vision complications.⁶ In addition, not all myopes have access to corrections for their blurry vision. Uncorrected refractive error (including myopia, hyperopia, presbyopia, and astigmatism) accounted for over half of global vision impairment and nearly a

quarter of global blindness in 1990 and 2010.⁷ It has also been estimated that uncorrected myopia is associated with an annual global potential productivity loss of \$244 billion.⁸

Atropine for myopia control

Clearly, action must be taken to counter the myopia epidemic. To this end, there have been renewed efforts to develop and prescribe treatments for myopia control (treatments to prevent or slow the progression of myopia in children). To date, atropine eye drops have shown the greatest effectiveness in reducing myopia progression, although the exact mechanism of atropine's anti-myopia effect remains unclear.⁹

Historically, daily 1% atropine has successfully been used for myopia control, but optometrists and ophthalmologists have been hesitant to prescribe it due to its significant ocular side effects (including increased sensitivity to bright light and blurry near vision).⁹ In addition, cessation of 1% atropine treatment leads to a "rebound" effect in which myopia rapidly progresses.¹⁰ However, following ATOM 2, a 2-year trial of 0.5%, 0.1%, and 0.01% atropine in Singapore¹¹, 0.01% atropine gained traction as a myopia control treatment. The trial demonstrated that when used for an extended (>1 year) period, 0.01% atropine achieved clinically significant reduction of myopia progression while minimizing ocular side effects and eliminating the rebound effect. Today, daily 0.01% atropine is commonly prescribed for myopia control in practice.

However, 0.01% atropine is not a universal solution for myopia control. Studies have shown that as atropine concentration decreases, the number of "nonresponders" (children who continue to experience high myopia progression despite atropine treatment) increases.^{11,12} In addition, although the results of meta-analyses suggest little difference in the efficacy of high (0.5-1%), moderate (>0.01-<0.5%), and low (0.01%) atropine concentrations^{13,14}, a recent trial of

three low atropine concentrations (0.05%, 0.025%, and 0.01%) found that efficacy increased with concentration, at least over a short-term (1-year) period.¹⁵ These conflicting results leave open questions about the relationship between atropine concentration and myopia control efficacy, what the optimal atropine dosing regimen looks like, and how this optimal regimen might vary between individuals.

A good starting point in our search for the optimal atropine dosing regimen for myopia control is to investigate and characterize the relationship between topical atropine concentration and patterns of atropine accumulation in the eye. Understanding how concentration affects active atropine levels in the eye over time with chronic (daily) atropine treatment can provide important direction in choosing what dosing regimens to evaluate next in a clinical setting.

Atropine's effects on pupil diameter, pupil response, and accommodative tone

Atropine is a competitive nonselective muscarinic antagonist; it irreversibly binds muscarinic receptors and blocks the activity of acetylcholine. Muscarinic receptors are present throughout the human eye, including the iris sphincter (which controls pupil constriction) and the ciliary muscle (which controls accommodation, or adjustment of the eye to focus on near objects). Atropine blocks contraction of the iris sphincter (Figure 1) and the ciliary muscle, thereby inducing mydriasis (pupil dilation and reduced pupil constriction in response to light) and cycloplegia (reduced accommodative tone). Patients receiving atropine eye drops may experience mydriasis as increased sensitivity to bright light and cycloplegia as blurry near vision. The higher the atropine concentration, the greater are these side effects.¹⁶ Thus, to evaluate the relationship between atropine concentration and patterns of atropine accumulation in the eye, these side effects—affecting pupil diameter, pupil response, and accommodative tone—have potential utility as biomarkers for active atropine levels in the eye.

In a 2018 study in humans¹⁷, researchers compared the acute ocular effects of three low atropine concentrations—0.01%, 0.005%, and 0.001%—by tracking changes in pupil diameter and accommodative function in response to a single drop of atropine over the course of the following day. They found that these outcome measures were indeed sensitive to concentration, and noted the need for further studies to see if these measures would also show changes over time with chronic (daily) treatment.

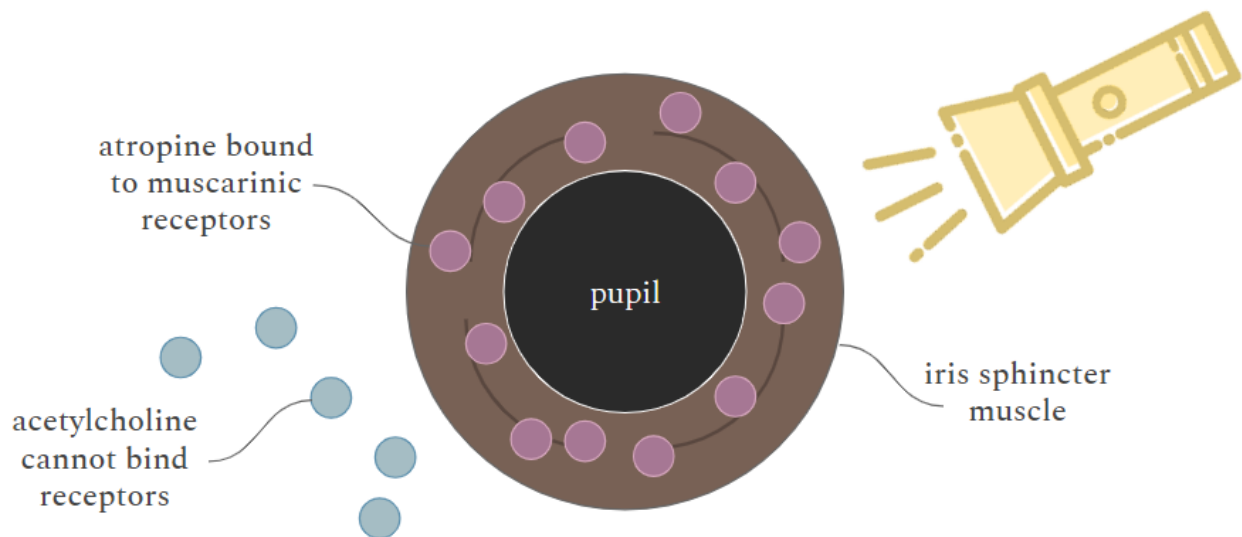


Figure 1. Atropine's action on the iris sphincter. Illumination stimulates the release of acetylcholine from parasympathetic nerve terminals. Normally, acetylcholine binds to muscarinic receptors on the iris sphincter and stimulates pupil constriction; however, if atropine occupies all receptors (as in the case illustrated), the pupil will not constrict.

The guinea pig model

The guinea pig is an increasingly popular mammalian model for myopia, in part due to its cooperative nature and relatively large eyes, both of which make it a suitable subject for studies of eye growth.¹⁸ For studies of atropine in particular, guinea pigs serve as an excellent model since their eyes share several relevant characteristics with human eyes. Firstly, muscarinic receptors are present throughout the guinea pig eye, including the iris and ciliary body.¹⁹

Secondly, guinea pigs exhibit accommodative ability.^{20,21} Lastly, pilocarpine—a muscarinic agonist—stimulates accommodation in guinea pigs and induces a myopic shift in measured refractive error.^{20,21}

Taken together, these characteristics mean that in response to topical atropine treatment, guinea pigs are likely to demonstrate changes in pupil diameter, pupil response, and accommodative tone similar to those that might be seen in humans.

Study objectives

This longitudinal randomized crossover study in guinea pigs monitored variations in pupil diameter, pupil response, and pilocarpine-induced change in refractive error (a measure of accommodative tone) over time to track saturation and recovery from daily 0.1% and 0.05% topical atropine treatment. We sought to answer the following questions:

1. Do variations in pupil diameter, pupil response, and/or pilocarpine-induced change in refractive error differ with atropine concentration? In other words, do these measures have utility as biomarkers for active atropine levels in the eye?
2. How does concentration affect the speed at which peak atropine saturation of muscarinic receptors is reached, and—following cessation of treatment—the speed at which atropine's effects drop to undetectable levels?
3. How does concentration affect the magnitude of atropine's effects at the time of peak muscarinic receptor saturation?

METHODS

Animals

Four pigmented guinea pigs (*Cavia porcellus*, Elm Hill strain) were included in the study over a period of nine months (June 2018 to March 2019). All guinea pigs were kept in the animal facilities of the University of California, Berkeley. They were housed in standard guinea pig cages under 12 h light/12 h dark cycle. All procedures were approved by the Animal Care and Use Committee at the University of California, Berkeley and met the ARVO resolution for care and use of laboratory animals.

Treatment

A randomized crossover study design was used (Figure 2). Guinea pigs were randomly assigned to one of two atropine regimen sequences: atropine 0.1% followed by atropine 0.05%, or atropine 0.05% followed by atropine 0.1%. Each atropine regimen consisted of a treatment phase (days 1-14) and a recovery phase (days 15-28). A single drop of atropine was administered daily during the treatment phase; no atropine was administered during the recovery phase. Animals received atropine in their right eye only. Atropine sulfate 0.1% and 0.05%, both preserved with benzalkonium chloride 0.01%, were prepared by San Diego Optimum Compounding Pharmacy, Poway, CA.

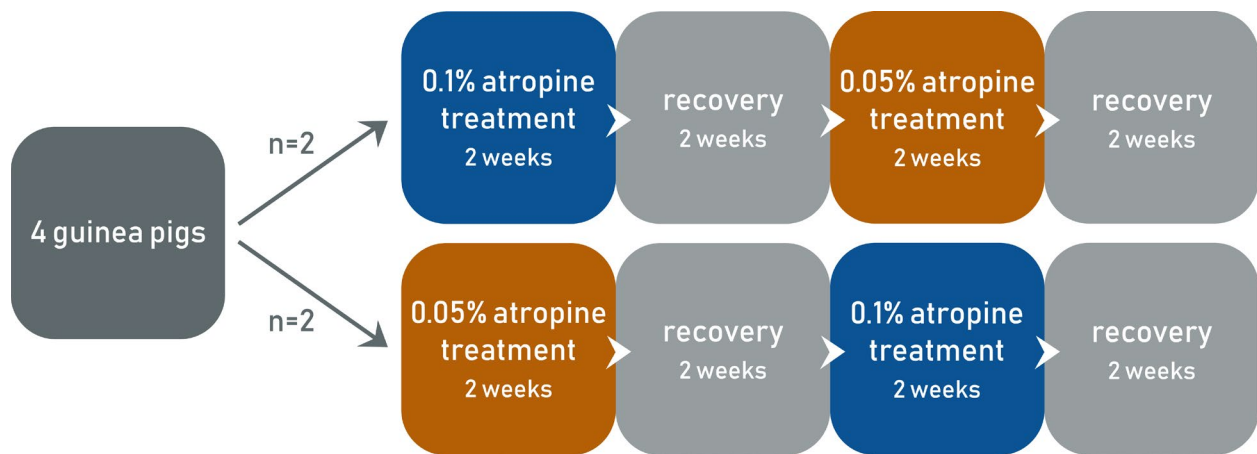


Figure 2. Study design and subject assignment to study arms.

Measurements

All measurements were performed by the same examiner, under the same ambient lighting conditions, on both the right and left eyes. Guinea pigs were alert and seated on a lab bench for all measurements. Pupil diameter and pupil response were measured monocularly using an automated pupillometer (NeuroOptics, Irvine, CA). A +16 diopter lens was attached to the front of the pupillometer to magnify and thus enable automatic recognition of the guinea pig pupil. In all cases, 3 measurements (of pupil diameter and pupil response) were taken and averaged. Refractive error was measured by retinoscopy. If guinea pigs were due to receive atropine, atropine was administered after all measurements were completed.

Baseline measurements were taken at the start of each atropine regimen, at least 1 day prior to the start of the treatment phase. At baseline, “complete measurements” were taken. The “complete measurements” sequence was as follows: pupillometry, retinoscopy, one drop pilocarpine hydrochloride 4% instillation in the right eye, 6-minute wait time, pupillometry, retinoscopy. As pilocarpine will bind to those muscarinic receptors left unoccupied by atropine, the magnitude of the difference between pre-pilocarpine and post-pilocarpine measurements will reflect the degree of atropine saturation of muscarinic receptors, with maximum saturation indicated by minimal response to pilocarpine.

The above pilocarpine protocol was based on a previous study in guinea pigs²⁰ in which researchers examined the ocular effects of one drop of topical pilocarpine 2%. They observed reductions in pupil diameter in all animals (n=8) 6 minutes after instillation. Myopic shifts in refractive error were also observed in all animals (n=5) after 10 minutes, although there was considerable inter-subject variability in when these myopic shifts reached their maximums. From these data, we selected a 6-minute wait time following the instillation of one drop of pilocarpine

4% before repeating our pupillometry-retinoscopy measurement sequence. Note that inter-subject variability as reported in the previous study would be considered less of a problem in the current study, as data analysis is focused on within-subject comparisons.

During the treatment phase of each atropine regimen (days 1-14), measurements were taken twice a week, on days 4, 7, 11, and 14. On days 4 and 11, pupillometry alone was performed. On days 7 and 14, “complete measurements” were taken.

During the recovery phase (days 15-28), measurements were taken three times each week, on days 16, 18, 21, 23, 25, and 28. On days 16 and 23, pupillometry alone was performed. On days 18 and 25, the following measurement sequence was performed: pupillometry, one drop pilocarpine hydrochloride 4% instillation in the right eye, 6-minute wait time, pupillometry. On days 21 and 28, “complete measurements” were taken.

Data analysis

All data were analyzed using Excel 2013 (Redmond, WA) and Stata 15 (College Station, TX).

Analysis of pupil response data for each regimen

“Day of atropine saturation of muscarinic receptors” was defined as the day on which a guinea pig recorded its least pupil constriction in response to light, expressed in percentage terms. If a guinea pig recorded the same minimum pupil response on two different days, the days were averaged to estimate the “day of saturation.” Wilcoxon signed-rank tests were conducted to evaluate atropine concentration-related differences in: a) the timing of saturation (“day of saturation”), and b) the percent pupil constriction on “day of saturation.”

“Day of recovery” was defined as the day on which pupil constriction in response to light returned to its previously recorded baseline value, expressed in percentage terms. If a guinea pig had not yet reached baseline pupil constriction on a given measurement day and then surpassed baseline pupil constriction on the following measurement day, these two days were averaged to estimate the “day of recovery.” If a guinea pig never fully recovered its baseline pupil response, day 28 was used as the “day of recovery.” Again, a Wilcoxon signed-rank test was conducted to evaluate atropine concentration-related differences in the timing of recovery (“day of recovery”).

Analysis of pupil diameter data

Analysis was limited to light-induced miotic pupil diameter, i.e., the smallest pupil diameter recorded in response to light (given by the pupillometer as “end” pupil diameter). “Day of saturation” was defined as the day on which a guinea pig recorded its largest pupil diameter. Wilcoxon signed-rank tests were conducted to evaluate atropine concentration-related differences in: a) the timing of saturation (“day of saturation”), and b) the percent change in pupil diameter from baseline to “day of saturation.”

“Day of recovery” was defined as the day on which a guinea pig recovered its baseline pupil diameter. A Wilcoxon signed-rank test was conducted to evaluate any atropine concentration-related differences in the timing of recovery (“day of recovery”).

Analysis of refractive error data

“Day of saturation” was defined as the day on which a guinea pig recorded its least difference in refractive error before versus after pilocarpine instillation. Wilcoxon signed-rank tests were conducted to evaluate any atropine concentration-related differences in: a) the timing

of saturation (“day of saturation”), and b) the pilocarpine-induced change in refractive error (pre-pilocarpine refractive error – post-pilocarpine refractive error) on “day of saturation.”

“Day of recovery” was defined as the day on which a guinea pig’s response to pilocarpine returned to its baseline response pattern. A Wilcoxon signed-rank test was conducted to evaluate atropine concentration-related differences in the timing of recovery (“day of recovery”).

Analysis of agreement between measures of saturation

Kendall’s W was calculated to evaluate concordance between the three measures of atropine saturation of muscarinic receptors (pupil response, pupil diameter, and refractive error) in their ordering of “day of saturation,” for all guinea pigs and both atropine concentrations.

Kendall’s W was similarly calculated to evaluate concordance in estimating “day of recovery.”

RESULTS

For the first treatment phase, baseline pupil constriction in response to light ranged from 5.33 to 11%; baseline pupil diameter ranged from 7.13 to 8.87 mm (as measured through a +16 diopter lens); baseline pilocarpine-induced change in refractive error ranged from 0.5 to 1.25 diopters. For the second treatment phase, baseline pupil constriction in response to light ranged from 9.67 to 16.67%; baseline pupil diameter ranged from 7.23 to 8.27 mm (as measured through a +16 diopter lens); baseline pilocarpine-induced change in refractive error ranged from 0.75 to 1.5 diopters. Pairwise comparison showed no significant differences in baseline pupil response ($p = 0.198$), pupil diameter ($p = 1$), or pilocarpine-induced change in refractive error ($p = 0.092$) recorded before starting the first treatment phase versus before starting the second treatment phase.

Time to saturation and recovery

Atropine concentration did not influence time to saturation (Table 1). Across measures of receptor saturation by atropine, mean estimates of “day of saturation” ranged from day 9.75 to 13.125 with 0.1% atropine and from day 9 to 14 with 0.05% atropine. On average, guinea pigs took longer to recover from 0.05% atropine treatment, but pairwise comparison showed that this trend did not reach significance (Table 2). Mean estimates of “day of recovery” ranged from day 21 to 22.75 with 0.1% atropine, compared to day 23.75 to 24 with 0.05% atropine. Except for one guinea pig which did not recover its pupil response by day 28 following 0.1% atropine treatment, all guinea pigs recovered by day 28 according to all three measures of saturation under both atropine regimens.

On the group level, the three measures of receptor saturation by atropine generally gave similar average times to saturation (Table 1) and recovery (Table 2). For both atropine concentrations, the pupil response measure gave mean estimates of “day of saturation” between day 10 and 11, and the pupil diameter measure gave mean estimates between day 9 and 10. Compared to these two measures, the refractive error measure gave delayed mean estimates of “day of saturation”: between day 13 and 14. The mean pupil response-based and pupil diameter-based estimates of “day of recovery” were between day 21 and 24, and the mean refractive error-based estimates were between day 22 and 24. Overall, there was fair agreement between measures in their estimates of “day of saturation” (Kendall’s W of 0.270) and very strong agreement between measures in their estimates of “day of recovery” (Kendall’s W of 0.829).

Table 1. Mean “day of saturation” according to three potential biomarkers for active atropine. Atropine was administered daily for two weeks. Pairwise comparison showed no significant difference in time to saturation between 0.1% and 0.05% atropine regimens according to any of the three atropine measures.

Measure of receptor saturation	0.1% atropine “day of saturation”	0.05% atropine “day of saturation”	P-value
Pupil response (pupil constriction in response to light)	10.625	10.25	0.578
Pupil diameter (change in light-induced miotic pupil diameter relative to baseline)	9.75	9	0.708
Refractive error (pilocarpine-induced change in refractive error)	13.125	14	0.842

Table 2. Mean “day of recovery” according to three potential biomarkers for active atropine. Daily atropine treatment was ceased after day 14. Pairwise comparison showed no significant difference in time to recovery between 0.1% and 0.05% atropine regimens according to any of the three measures.

Measure of receptor saturation	0.1% atropine “day of recovery”	0.05% atropine “day of recovery”	P-value
Pupil response (pupil constriction in response to light)	21	24	0.273
Pupil diameter (change in light-induced miotic pupil diameter relative to baseline)	21	23.75	0.162
Refractive error (pilocarpine-induced change in refractive error)	22.75	24	0.157

Degree of saturation

Pupil response

With the higher (0.1%) concentration atropine, a greater degree of receptor saturation was achieved ($p = 0.034$), as defined as lower percent pupil constriction in response to light on “day of saturation.” With 0.1% atropine treatment, the average percent pupil constriction on “day of saturation” was 0.92%. With the lower 0.05% atropine treatment, the average percent pupil constriction on “day of saturation” was 3.33%.

Day-by-day comparison showed a clear trend that 0.1% atropine resulted in less pupil constriction in response to light than 0.05% atropine on each day of the treatment phase, as well as on days 16 and 18 of the recovery phase (Figure 3), indicating greater receptor saturation.

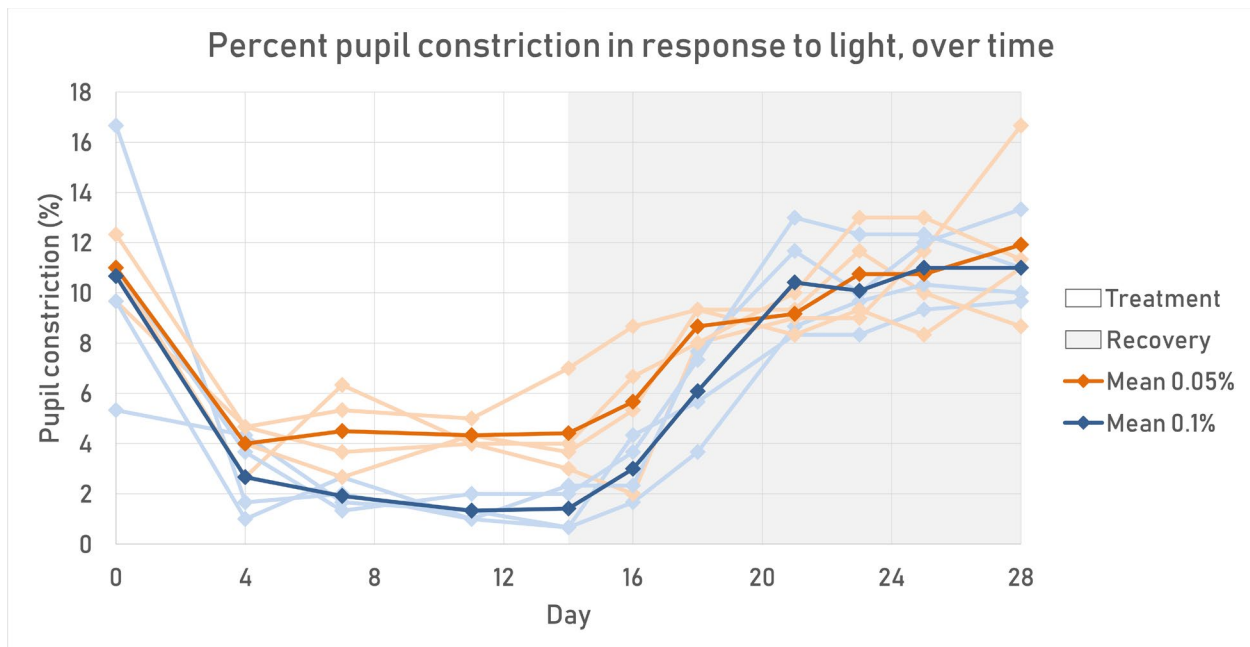


Figure 3. Pupil constriction in response to light, recorded over the course of each atropine regimen. Daily atropine treatment was ceased after day 14. Paler lines represent individual guinea pig data. During and immediately following the treatment phase, pupil responses generally appeared lower with 0.1% atropine than with 0.05% atropine.

Light-induced miotic pupil diameter

Atropine concentration did not significantly affect the degree of receptor saturation as defined as the percent change in pupil diameter from baseline to “day of saturation” ($p = 0.233$). With 0.1% atropine treatment, the average percent change in pupil diameter from baseline to “day of saturation” was 19.3%, and with 0.05% atropine treatment, the comparable figure was 19.1% (Figure 4).

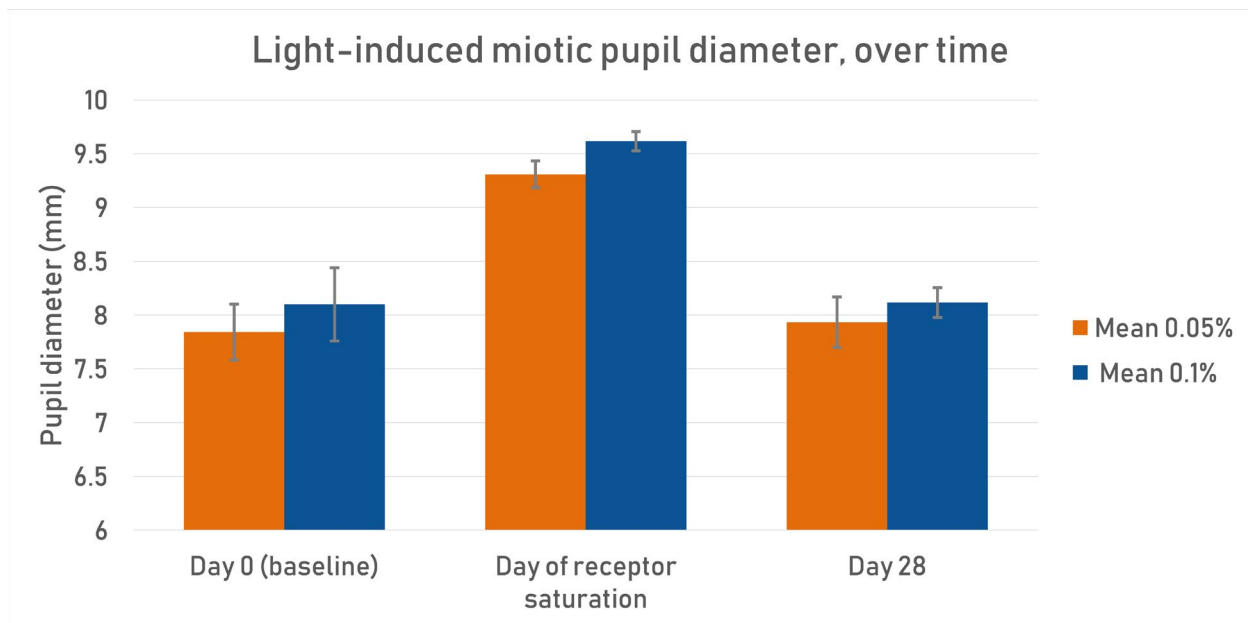


Figure 4. Light-induced miotic pupil diameter (as measured through a +16 diopter lens), recorded over the course of each atropine regimen. Pairwise comparison showed no significant atropine concentration-related difference in the percent change in pupil diameter from baseline to “day of saturation.”

Pilocarpine-induced change in refractive error

Atropine concentration also did not significantly affect the degree of saturation as defined in terms of the pilocarpine-induced change in refractive error on “day of saturation” ($p = 0.099$). The average pilocarpine-induced change in refractive error on “day of saturation” was 0.0625 diopters with 0.1% atropine treatment, compared to 0.375 diopters with 0.05% atropine treatment (Figure 5).

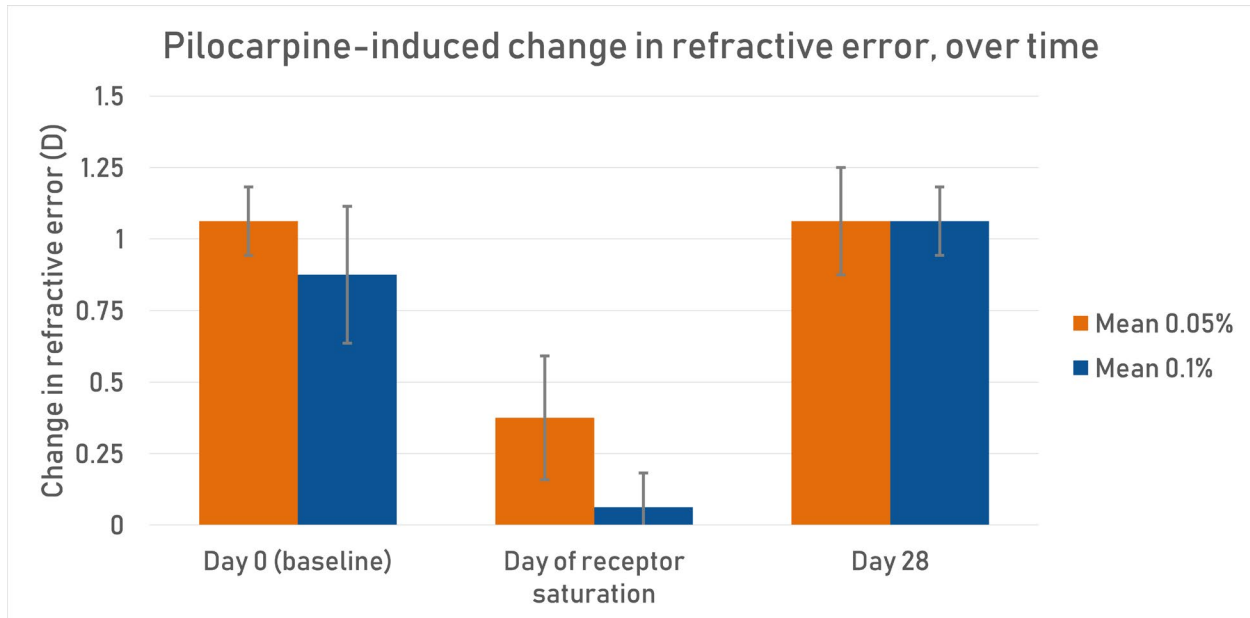


Figure 5. Pilocarpine-induced change in refractive error, recorded over the course of each atropine regimen. Pairwise comparison showed no significant atropine concentration-related difference in the pilocarpine-induced change in refractive error on “day of saturation.”

DISCUSSION

This randomized crossover study in guinea pigs tracked muscarinic receptor saturation and recovery from daily topical dosing with two different concentrations of atropine. In agreement with past human studies linking higher concentrations of atropine to greater side effects^{16,17}, we found that chronic treatment with the higher concentration atropine led to a greater degree of muscarinic receptor saturation in the guinea pig eye. This was indicated by a significantly greater reduction in pupil constriction in response to light with the 0.1% atropine treatment than with the 0.05% atropine treatment. This is in contrast to light-induced miotic pupil diameter and pilocarpine-induced change in refractive error, neither of which showed significant atropine concentration-dependent differences.

Interestingly, while the higher concentration atropine led to a greater degree of saturation, the time to saturation did not vary with atropine concentration. Perhaps more interestingly, all

three measures of receptor saturation hinted at faster recovery from the 0.1% atropine treatment than the 0.05% atropine treatment, although this pattern did not reach significance. This finding—that increasing the concentration of atropine did not increase the duration of saturation, and if anything, may have decreased the duration of saturation—opens up the possibility of compensatory and concentration-dependent upregulation of muscarinic receptors, as reported in other pharmacological studies of atropine.^{22,23}

Future directions

The demonstrated sensitivity of pupil response to atropine concentration in guinea pigs raises the possibility of its use as a biomarker for active atropine levels in the eye. Thus these findings warrant follow-up studies in humans. If humans do show similar atropine concentration-dependent pupil responses, it may be possible to quantify individual sensitivity to atropine in terms of individual dose-response curves. Assuming such pupil data predict sensitivity to the myopia control effects of atropine, they could then be used to estimate the optimal atropine concentration to prescribe individual patients for myopia control, at least as a starting point. This approach to dosing would be more personalized and efficient than the current approach, which is to start with 0.01% atropine, then prescribe higher concentrations only if myopia progression is not sufficiently slowed based on measurements made some 12 months or more later.

Since atropine concentration did not seem to influence the time to saturation or recovery, it is possible that the frequency of dosing may also not influence time to saturation or recovery. (Reducing concentration and reducing dosing frequency both effectively lower the amount of atropine being administered.) It is important to note that with daily dosing, ocular muscarinic receptors receive consistent influxes of atropine; thus, even as atropine-bound receptors are lost and replaced, “new” atropine continues to saturate “new” receptors. In contrast, with less

frequent dosing, the degree of saturation may decrease over time as atropine-bound receptors are lost and replaced between doses. However, it is also possible that the degree of saturation will be maintained over time if atropine which has been taken up by ocular melanin (in the iris and choroid) is gradually released onto “new” receptors. Past studies have reported atropine fixation by melanin^{24,25}, longer atropine retention in pigmented versus albino rabbit irises²⁶, and prolonged mydriatic effects following topical atropine instillation in pigmented versus albino rabbits^{26,27}, suggesting that the slow release of melanin-bound atropine onto muscarinic receptors prolongs atropine’s ocular effects.

In order to examine the influence of atropine fixation and release by melanin on the degree of muscarinic receptor saturation by atropine over time, future studies might evaluate the effect of dosing frequency (for example, daily versus every-other-day or biweekly dosing) on receptor saturation and recovery from topical atropine treatment. Ultimately, if it can be demonstrated that less frequent dosing does not result in reduced myopia control efficacy, the dosing frequency prescribed to patients in practice could be lowered. This would minimize side effects and reduce patients’ total drug exposure.

Strengths and limitations

One of this study’s greatest strengths was its longitudinal design with frequent measurements, allowing us to closely track intraocular atropine accumulation and its decline over time. To our knowledge, our study is also the first to evaluate the effect of concentration on the ocular side effects of chronic atropine treatment in guinea pigs. Simultaneously tracking three well-known side effects of atropine—impaired pupil response, pupil dilation, and cycloplegia—allowed for direct comparison of the viability of these side effects as biomarkers for active atropine levels in the eye.

Another strength of this study was its randomized crossover design, allowing us to make within-subject comparisons. The fact that pupil responses differed with atropine concentration *on an individual level* further supports our recommendation to look into pupil response as a tool for personalizing atropine dosage.

The greatest limitation of this study was its small sample size. It is possible that guinea pigs indeed recover faster from 0.1% atropine treatment than 0.05% atropine treatment, and/or that light-induced miotic pupil diameter and/or pilocarpine-induced change in refractive error are in fact sensitive to atropine concentration, and we simply did not have the power to detect such differences. In order to determine whether recovery from the ocular effects of atropine indeed takes longer with 0.05% concentration than with 0.1% concentration, this study should be replicated with a larger sample size. In addition, it is important to note that since refractive error was measured less often than pupil response and pupil diameter (weekly versus every 2-4 days), we may have been less accurate in our estimates of when pilocarpine-induced changes in refractive error reached their minimums and fully recovered.

A final limiting factor was our adaptation of a human pupillometer for use on guinea pigs. Although use of a +16 diopter lens allowed for successful recognition of the guinea pig pupil, we had no way of standardizing the distance between the lens and the guinea pig eye. Visually, we checked to make sure that the image of the eye on the pupillometer screen was sharp and in focus (indicating proper distance) during each measurement. However, slight variations in the distance and angle of approach could have introduced small errors and contributed to variability in our measured pupil responses and pupil diameters. Nonetheless, using the average of 3 measurements and having the same examiner perform all measurements helped to reduce variability.

Conclusion

This study of chronic topical atropine treatment in guinea pigs showed that atropine concentration influenced the degree of receptor saturation in the guinea pig eye, but not the time to saturation or recovery. Logical next steps include: a) scaling up the study to improve the statistical power of the data, and b) evaluating the effect of dosing frequency on the time and degree of receptor saturation and recovery from atropine treatment. A longer-term goal, based on the premise that atropine-induced changes in pupil response can be used as a biomarker for active atropine levels, is to validate these findings in the human eye. Doing so could allow practitioners to use pupil responses to customize myopia control treatment for individual patients.

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