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Combined 0.01% atropine with orthokeratology in childhood myopia control (AOK) study: A 2-year randomized clinical trial

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ABSTRACT

Background: To investigate whether combining 0.01% atropine with orthokeratology (AOK) has a better effect in retarding axial elongation, compared with orthokeratology alone (OK) over two years.
Methods: A total of 96 Chinese children aged six to < 11 years with myopia (1.00 – 4.00 D, inclusive) were randomized into either the AOK or OK group in a 1:1 ratio.
Axial length (the primary outcome), and secondary outcomes (e.g. pupil size and choroidal thickness) were measured at 1-month and at 6-monthly intervals after commencement of treatment.
Results: Both intention-to-treat and per-protocol analyses showed significantly slower axial elongation in the AOK group than OK group over two years ($P = 0.008$, $P < 0.001$, respectively). AOK subjects had statistically slower axial elongation (adjusted mean [standard error], 0.17 [0.03] mm vs 0.34 [0.03] mm, $P < 0.001$), larger increase in mesopic (0.70 [0.09] mm vs 0.31 [0.09] mm, $P = 0.003$) and photopic pupil size (0.78 [0.07] mm vs 0.23 [0.07] mm, $P < 0.001$), and greater thickening of the choroid (22.6 [3.5] μm vs −9.0 [3.5] μm, $P < 0.001$) than OK subjects over two years. Except for a higher incidence of photophobia in the AOK group ($P = 0.006$), there were no differences in the incidence of any other symptom or adverse events between the two groups. Slower axial elongation was associated with a larger increase in the photopic pupil size and a greater thickening in the choroid in the AOK group.
Conclusions: Slower axial elongation following 2-year AOK treatment may result from increased pupil dilation and a thickening in the choroid observed in the AOK group.

1. Introduction

The prevalence of myopia and high myopia have reached alarming rates in Chinese adolescents aged 16 to 18 years, reaching 84.8% and 19.3%, respectively [1]. It is estimated that by 2050, 84% of Chinese aged between 3 and 19 years would be myopic [1]. To limit childhood myopia progression and, consequently, reduce the risk of myopia-associated sight-threatening ocular pathologies [2–5], a range of optical and pharmacological interventions have been investigated [6,7]. To date, except for the use of 1% atropine [8], no single intervention has been shown to be effective in totally inhibiting myopia progression in terms of either equivalent spherical refraction (SER) or axial elongation [6,7]. However, the use of 1% atropine, along with other concentrations (i.e. 0.5%, 0.1%), is not acceptable as a mainstream myopia control therapy due to significant side-effects during treatment [8,9] and a strong rebound effect after discontinuation [10,11]. Despite well-tolerated side-effects, the effect of low concentration atropine (i.e. 0.05%, 0.025%, and 0.01%) in slowing axial elongation decreased with reduced concentration (i.e. slowed by 51%, 29%, and 12%, respectively, compared with the placebo treatment over one year) [12]. Of the optical interventions for myopia control, orthokeratology (*ortho*-k) was ranked as the most effective [6], being able to reduce axial elongation by 43% – 63% compared to wearing single-vision spectacles [13–18] or soft contact lenses over two years [19].

Combination therapy has been suggested as an approach to improve treatment efficacy in retarding axial elongation [7]. The underlying rationale for combination of atropine and *ortho*-k is that an additive effect may exist, as different mechanisms of action are believed to be

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involved in these two interventions for myopia control [20–23]. As use of 0.01% atropine has minimum side-effects [24,25], several studies have investigated the use of combined 0.01% atropine and *ortho*-k for myopia control [26–30]. In a randomized study conducted by Kinoshita et al., an additive effect of this combined treatment was observed over two years in children with an initial SER of -1.00 to -3.00 D, but not in those with moderate to high initial SER (-3.01 to -6.00 D) [26]. In a retrospective study, Chen et al. found that adding 0.01% atropine to *ortho*-k for two years for children with rapid axial elongation ($\geq$ 0.30 mm during initial 1-year *ortho*-k), did not result in slower axial elongation compared with similar fast progressors that received *ortho*-k alone continuously for three years [27]. Notably, combined therapy only commenced after 3-month [26] or 1-year [27] treatment of *ortho*-k in these studies, at which point, the use of 0.01% atropine was added to the test group.

The Atropine combined with Orthokeratology (AOK) study was conducted to explore whether there is an additive effect in retarding axial elongation if 0.01% atropine is used in conjunction with *ortho*-k over two years, employing *ortho*-k alone as a comparator [30]. As the use of 0.01% atropine alone failed to retard myopic progression in terms of axial elongation over one year (placebo-controlled) [12] or two years (using historical controls) [9], a treatment group using 0.01% atropine was not included [30]. The primary outcome was the change in axial length (i.e. axial elongation), with changes in pupil size and choroidal thickness included as secondary outcomes.

2. Methods

2.1. Study design

The 2-year AOK study was an interventional, single-masked, randomized study (ClinicalTrials.gov number, NCT02955927), which was designed to achieve 80% power to detect a minimum difference of 0.18 mm in axial length over two years with 5% level of significance [30]. Using the within group standard deviation (SD) of 0.25 mm from the ROMIO study [14], at least 48 subjects (24 in each group) were required at completion [30]. Ethical approval was obtained from the Human Subject Ethics Subcommittee of the School of Optometry of The Hong Kong Polytechnic University (PolyU) and the Institutional Review Board of The University of Hong Kong (HKU) /Hospital Authority Hong Kong West Cluster. A certificate for the clinical trial/medicinal test was obtained from the Pharmacy and Poison Board, Department of Health of Hong Kong. All children provided assent and parents provided informed consent before participation, with all procedures following the tenets of the Declaration of Helsinki.

2.2. Subjects and randomization

The inclusion and exclusion criteria were elaborated previously [30]. In brief, children of Chinese ethnicity aged six to $<$ 11 years, with normal ocular health other than myopia (1.00 – 4.00 D, inclusive), no history of myopia control treatment, and documented myopic progression in SER of at least 0.50 D in the past one year were enrolled [30].

2.3. Intervention

Treatment in the AOK group consisted of instillation of one drop of preservative-free 0.01% atropine (Aseptic Innovative Medicine Co., Ltd., Taiwan) into each eye, 10 min before nightly wear of 4-zone *ortho*-k lenses (KATT BE Free Lens, Precision Technology Services, Vancouver, B.C., Canada), while subjects in the OK group only wore *ortho*-k lenses nightly.

2.4. Masking and treatment compliance

All subjects and investigators who performed the follow-up were unmasked about the assigned treatment. A masked examiner randomized eligible subjects into either the AOK or OK group in a 1:1 ratio, using a commercial spreadsheet random number generator (Excel; Microsoft, Redmond, Washington, USA). A masked investigator who was not involved in the follow-up of subjects measured the axial length. AOK subjects were required to apply 0.01% atropine nightly and return empty vials to investigators at follow-up visits. All subjects were instructed to wear *ortho*-k lenses nightly for at least eight hours, except during ill health and ocular discomfort. Compliance with *ortho*-k lens wear was assessed based on reports from parents and subjects, by calculating the rate of lens wear (total number of nights with lens wear/ total number of days during the study). The rate of using atropine eye drops in the AOK group was calculated (total numbers of returned empty vials/total number of days during the study).

2.5. Examination procedures

All subjects were required to attend cycloplegic examinations (data collection visits) at one month, and then every 6 months after commencement of the treatment at the Optometry Clinic of the School of Optometry of PolyU. Following the baseline cycloplegic assessment, subsequent data collection was carried out within $\pm$ 2 h of the measurement time of the baseline visit, to reduce the influence of diurnal variation in ocular parameters, particularly choroidal thickness [31–33]. Subjects in the AOK group were also required to attend 3-monthly ophthalmologist visits for atropine prescription and ocular health monitoring at the HKU eye clinic.

At each data collection visit, manifest subjective refractive error was measured using a trial frame before and after cycloplegia, following the principle of maximum plus for maximum visual acuity (VA). Unaided VA (UVA) and best-corrected VA (BCVA) were measured using high contrast (100%) Early Treatment Diabetic Retinopathy Study charts (Precision Vision, La Salle, Illinois, USA) under normal room lighting at a 4-meter distance. Non-cycloplegic pupil size was measured using the OPD-Scan III (Nidek, Gamagori, Japan) with an internal light source, under mesopic illuminance (3.5 lx), followed by photopic illuminance (125.6 lx) in a closed room with the lights off. During the pupil size measurement, subjects were required to fixate on the internal instrument target and were fogged to relax their accommodation. The first three measurements with a difference of $<$ 0.50 mm were averaged for analyses. With the optimal distance refraction in place, the amplitude of accommodation was measured three times, using the Royal Air Force Rule (Harlow, Essex, UK) (push up method) for each eye, which were later averaged for analysis.

Before cycloplegia, choroidal thickness was measured using the Spectralis SD-OCT (Heidelberg Engineering, Inc., Heidelberg, Germany) under high-speed scanning and enhanced depth imaging mode (i.e. six foveal centered 30-degree long radial line scans of each line consisting of 30 frames). Of the three baseline measurements, the one that demonstrated the highest quality, in terms of a quality index (at least 25 dB), served as the reference image for all follow-up scans. At subsequent data collection visits, using automatic real-time tracking, the first three measurements with a full choroidal image and a quality index over 25 dB were saved and later exported. Using customized software and manual correction (where appropriate) [34,35], horizontal scans of the choroid were semi-automated and used for analyses. Measurement of axial length was performed by a masked examiner using Zeiss IOLMaster (Carl Zeiss Meditec AG, Jena, Germany) at least 30 min after cycloplegia using two drops of 1% cyclopentolate administered 5 min apart. Composite readings based on five readings with a maximum difference of 0.02 mm and a signal-to-noise ratio above five, were used for analysis.

2.6. Data analysis

Data from the right eye of subjects were used for data analyses, using IBM SPSS Statistics for Windows (Version 25.0. IBM Corp., Armonk,

New York, USA). Intention-to-treat (ITT) analyses were performed by including all subjects who received randomization, using linear mixed models with unstructured covariance structure and restricted maximum likelihood estimation. Individual slope and intercept were included as random effects and an unstructured covariance matrix was used to control inter-subject variation. It was found that baseline age and SER were associated with axial elongation in children who underwent orthoky [14,36,37] or treatment of low-concentration atropine [38,39]. Thus, baseline age and SER were included as covariates. For ITT analyses, baseline parameters (age, sex, SER, pupil size, the amplitude of accommodation, and choroidal thickness), changes in parameters over two years (e.g. axial length, pupil sizes, amplitude of accommodation, and choroidal thickness), cycloplegic SER, and UVA, were compared between the two groups of subjects. This linear mixed model was also used to assess whether baseline parameters (age, sex, SER, pupil size, the amplitude of accommodation, and choroidal thickness) influenced axial

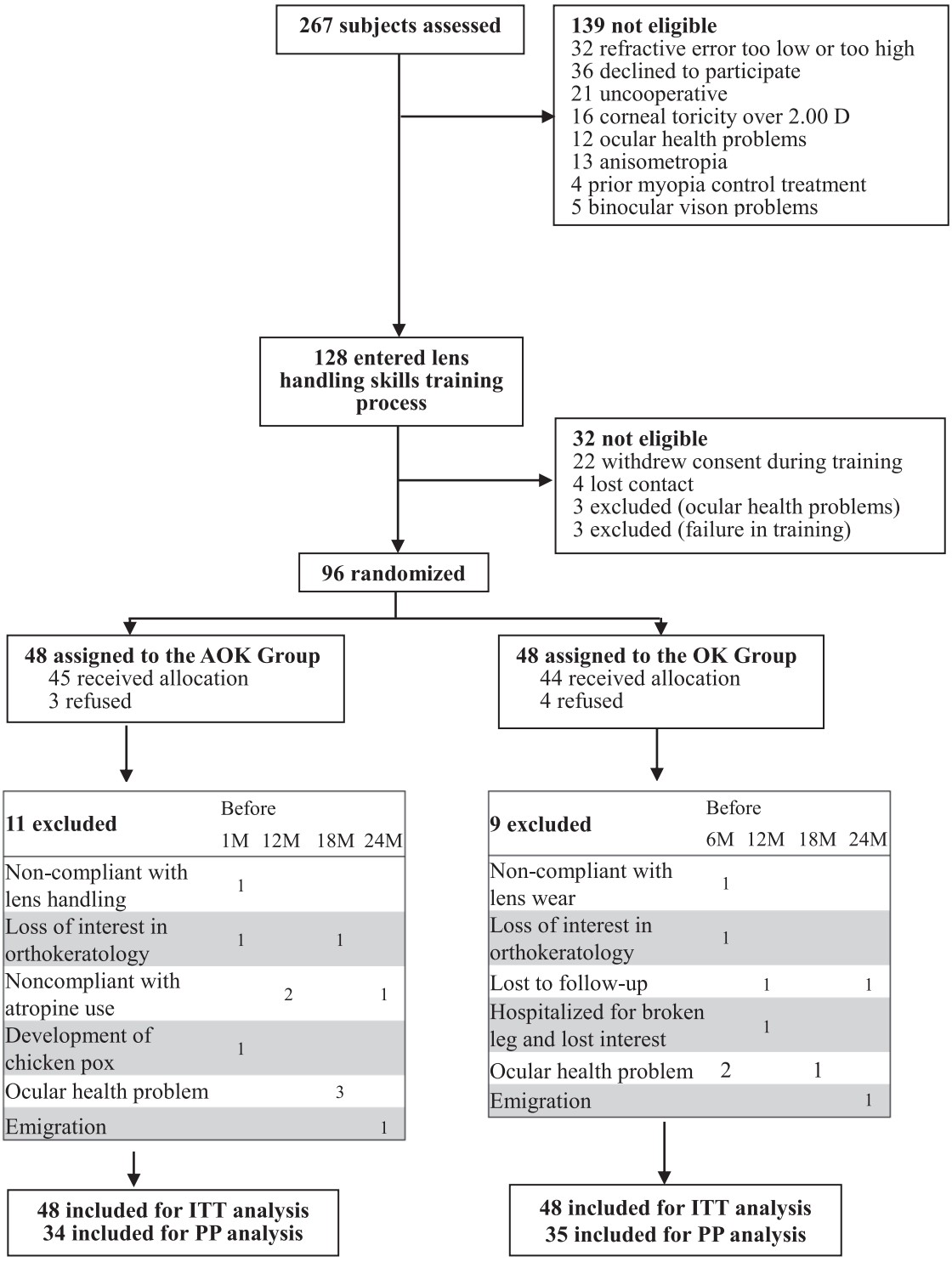

Fig. 1. Flow chart showing subject recruitment and dropouts.
AOK – Combined atropine with orthokeratology; OK – Orthokeratology alone; ITT – Intention-to-treat; PP – Per-protocol.

elongation in each group and all subjects (Model 1). If baseline parameters were significantly associated with axial elongation in Model 1, they would be adjusted in Model 2; otherwise, they were excluded. In Model 2, the effects of the post-treatment changes in pupil size, the amplitude of accommodation, and choroidal thickness on axial elongation were examined with the same modeling as Model 1.

For per-protocol (PP) analyses, data from subjects who completed the 2-year study were included; the normality of the data was explored using the Kolmogorov-Smirnov test; after a normal distribution was confirmed, unpaired t-tests were used to compare baseline age, SER, pupil size (mesopic and photopic), the amplitude of accommodation, and choroidal thickness between the two groups. Two-way repeated measures analyses of covariance (RM ANCOVA) were controlled for the effect of baseline age and SER, to compare parameters, including axial elongation, changes in pupil sizes and the amplitude of accommodation, cycloplegic SER, UVA, and changes in the choroidal thickness, between the two groups of subjects over two years; post hoc analyses using the Bonferroni correction were applied to examine between-group or between-visit differences, where appropriate; crosstab analysis was used to compare the gender ratio and the percentage of subjects reporting symptoms, including photophobia, halo, and itching, between the two groups; unpaired and paired t-tests were used to compare the 6-monthly axial length changes between the two groups and within each group, respectively. A P-value < 0.05 was considered statistically significant, except where multiple comparisons were made, in which case, a Bonferroni-adjusted P-value of < 0.013 (0.05/4) or 0.008 (0.05/6), where appropriate, was used to indicate significance.

3. Results

3.1. Subject profile

A total of 96 subjects were randomized (Fig. 1). After randomization, 89 subjects (45 AOK and 44 OK subjects) commenced the treatment, while 11 AOK and nine OK subjects were excluded at different stages of the study for various reasons (Fig. 1), resulting in a total of 69 subjects (34 AOK and 35 OK) completing the 2-year study.

3.2. Baseline characteristics

No significant differences in the baseline characteristics was observed between the two groups of subjects who received randomization (all $P > 0.05$) or who completed the 2-year study (all $P > 0.05$, Table 1). For subjects who completed the 2-year study, the mean rate of lens wear was 93% (95% confidence interval: 92% – 94%) for both groups; the mean rate of application of atropine eye drops was 93% (95% confidence interval: 92% – 94%).

Table 1
Demographics and baseline data (Mean ± SD) of subjects who completed the 2-year study.

	AOK (n = 34)	OK (n = 35)	P
Age (years)	9.2 ± 1.0	9.1 ± 1.2	0.513
Male/Female	15/19	15/20	0.916
Axial Length (mm)	24.56 ± 0.71	24.50 ± 0.92	0.795
SER (D)	-2.76 ± 0.88	-2.83 ± 1.01	0.745
Mesopic pupil size (mm)	6.48 ± 0.86	6.61 ± 0.82	0.517
Photopic pupil size (mm)	3.24 ± 0.31	3.29 ± 0.32	0.611
BCVA (logMAR)	-0.04 ± 0.05	-0.03 ± 0.05	0.418
Accommodation (D)	13.5 ± 1.9	12.8 ± 2.2	0.217
Choroidal thickness (μm)	244.2 ± 46.3	234.6 ± 47.0	0.398

AOK – Combined atropine with orthokeratology.
OK – Orthokeratology alone.
SER – Spherical Equivalent Refraction.
BCVA – Best Corrected Visual Acuity.
P – Probability value of unpaired t-test for differences between groups (Crosstab analysis was used to compare the gender ratio).

3.3. Axial elongation

For ITT analyses, when axial elongation was included as independent variable in the linear mixed model, the fixed effects of treatment and treatment by visit were significant ($P < 0.001$, $P = 0.008$, respectively). PP analyses showed that mean ± SD axial elongation over two years were 0.17 ± 0.19 mm and 0.35 ± 0.20 mm in the AOK and OK groups, respectively; the adjusted overall mean axial elongation in the AOK group was 0.18 mm less than that in the OK group over two years ($P < 0.001$, Table 2). A significant treatment by visit interaction was observed for axial elongation ($P = 0.003$, Table 2), and the main effect of time and treatment were significant (both $P < 0.001$). A significant difference in axial elongation between the two groups was only observed for the first 6-month period ($P < 0.001$, Table 3). In the AOK group, significantly less axial elongation was observed in the first 6-month period than that for the second, third, and fourth 6-monthly periods (all $P < 0.001$). In the OK group, significantly different axial elongation was only oberved between the first and the second 6-month periods ($P = 0.003$), but not between any other 6-month periods (all $P > 0.008$).

Linear mixed model (Model 1) showed that axial elongation was not associated with any of the baseline parameters in either group or pooled subjects (beta: -9.756 to -0.001, all $P > 0.05$). In the AOK group, axial elongation were negatively associated with the changes in photopic pupil size (beta: -0.025, $P = 0.031$; Model 2) and choroidal thickness (beta: -0.002, $P < 0.001$; Model 2), but not with either the changes in mesopic pupil sizes or changes in the amplitude of accommodation (beta: -0.003 to -0.002, $P = 0.709$, 0.267, respectively). Except for the change in choroidal thickness (beta: -0.002, $P < 0.001$; Model 2), no association was found between axial elongation and changes in any other parameters in the OK group (all $P > 0.05$).

3.4. Cycloplegic spherical equivalent refraction, visual acuity, and changes in pupil sizes, the amplitude of accommodation, and choroidal thickness

For PP analyses, significant differences in cycloplegic residual SER between the two groups were only observed at the 6-month and 18-month visits ($P = 0.004$, $P < 0.001$, respectively). However, both the actual and adjusted difference was clinically insignificant (< 0.50 D, Table 2). In the AOK group, there was a thickening in the choroid after treatment, which stabilized over the two years (adjusted mean: 20.0 – 22.9 μm, Table 2), given that no significant differences were observed between any of the two post-treatment visits (all $P > 0.05$). In the OK group, the adjusted changes in choroid thickness at the 18- and 24-month visits (adjusted mean of difference [SE]: -6.9 [3.7] μm, -9.0 [3.5] μm, respectively, Table 2) were significantly different from those at the 1-month visit ($P = 0.027$, 0.004, respectively). However, no significant difference in the adjusted changes in choroidal thickness was observed between the 1-month visit and the 6-, 12-month visits, respectively ($P = 0.460$, 0.103, respectively).

For both ITT and PP analyses, changes in mesopic and photopic pupil sizes in the AOK group were significantly greater than those in the OK group at all post-treatment visits (all $P < 0.05$); significantly greater changes (thickening) in choroidal thickness were observed in the AOK group than those in the OK group (all $P < 0.05$); no significant between-group differences in UVA, BCVA, or changes in the amplitude of accommodation (all $P > 0.05$).

3.5. Ocular symptoms and adverse events

Except for a higher percentage of subjects who suffered from photophobia (well-tolerated without complaints of causing inconvenience) in the AOK group than in the OK group ($P = 0.006$), there were no differences in the percentage of subjects experiencing any other symptom and adverse events between the two groups (Table 4). No severe adverse event (e.g. microbial keratitis) was observed. Three

Table 2
Mean [SE] ocular parameters and difference over two years in the two groups of subjects who completed the 2-year study, while controlling for the effect of baseline age and spherical refractive error.

Ocular parameters	Group	Mean [SE] 1 M	6 M	12 M	18 M	24 M	Group × visit interaction (F, *P*)	*P*' (12 M vs 24 M) AOK	OK
Axial elongation, mm	AOK	-0.05 [0.01]	-0.02 [0.02]	0.06 [0.03]	0.10 [0.03]	0.17 [0.03]	6.90, **0.003**	**< 0.001**	**< 0.001**
	OK	-0.01 [0.01]	0.07 [0.02]	0.18 [0.03]	0.25 [0.03]	0.34 [0.03]			
Difference		0.04 [0.01]	0.09 [0.02]	0.11 [0.04]	0.15 [0.04]	0.18 [0.05]			
P†		**< 0.001**	**< 0.001**	**0.003**	**0.001**	**< 0.001**			
Changes in photopic pupil, mm	AOK	0.73 [0.07]	0.55 [0.06]	0.60 [0.07]	0.65 [0.07]	0.78 [0.07]	1.50, 0.211	0.325	0.150
	OK	-0.02 [0.06]	-0.03 [0.06]	0.03 [0.07]	0.11 [0.07]	0.23 [0.07]			
Difference		0.75 [0.09]	0.58 [0.08]	0.58 [0.10]	0.54 [0.09]	0.56 [0.10]			
P†		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**			
Changes in mesopic pupil, mm	AOK	0.63 [0.06]	0.63 [0.07]	0.59 [0.08]	0.68 [0.09]	0.70 [0.09]	1.16, 0.328	0.999	0.287
	OK	0.08 [0.06]	0.11 [0.07]	0.14 [0.08]	0.11 [0.08]	0.31 [0.09]			
Difference		0.55 [0.09]	0.52 [0.10]	0.45 [0.11]	0.57 [0.12]	0.40 [0.13]			
P†		**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**	**0.003**			
Changes in the amplitude of accommodation, D	AOK	-0.8 [0.4]	-1.5 [0.4]	-1.2 [0.3]	-2.0 [0.4]	-2.2 [0.3]	1.28, 0.279	**< 0.001**	0.521
	OK	-0.6 [0.4]	-0.7 [0.4]	-0.8 [0.3]	-1.1 [0.4]	-1.2 [0.3]			
Difference		0.2 [0.5]	0.8 [0.5]	0.4 [0.5]	0.9 [0.5]	1.0 [0.5]			
P†		0.116							
Changes in choroidal thickness,* μm	AOK	20.0 [2.3]	17.4 [2.8]	21.8 [3.7]	22.9 [3.6]	22.6 [3.5]	8.38, **< 0.001**	0.999	0.962
	OK	5.5 [2.3]	0.1 [2.9]	-3.6 [3.8]	-6.9 [3.7]‡	-9.0 [3.5]‡			
Difference		9.5 [3.3]	17.2 [4.1]	25.4 [5.3]	29.8 [5.2]	31.6 [5.0]			
P†		**0.006**	**< 0.001**	**< 0.001**	**< 0.001**	**< 0.001**			
Cycloplegic SER, D	AOK	0.15 [0.07]	0.32 [0.08]	0.18 [0.08]	0.25 [0.08]	0.08 [0.08]	4.24, **0.004**	0.999	0.999
	OK	0.19 [0.07]	-0.01 [0.08]	0.03 [0.07]	-0.18 [0.08]	-0.09 [0.08]			
Difference		0.04 [0.09]	0.33 [0.11]	0.15 [0.11]	0.43 [0.11]	0.17 [0.11]			
P†		0.639	**0.004**	0.162	**< 0.001**	0.106			

AOK – Combined atropine with orthokeratology (n = 34 at each visit); OK – Orthokeratology alone (n = 35 at each visit); SER – Spherical Refractive Error; Bold – Indicates significance; SE – Standard Error; All changes were based on baseline data.
-ve Values indicate reduced/thinning; +ve Values indicate increase/thickening.
‡ Significantly different from 1 M.
P – Probability value of RM ANCOVA, with post hoc analyses (*P*†) for differences between groups over time; *P*' – Post hoc analyses for differences in parameters between 12-month and 24-month visits in each group; *– comparison of the AOK (n = 33) and OK group (n = 32) due to missing data of 4 subjects (1 AOK and 3 OK).

Table 3
Six-monthly increase in axial length in the two groups of subjects over two years.

6-monthly increase	Mean ± SD AOK n = 34	OK n = 35	Difference Mean [SE]	95% CI	*P* #
First	-0.02 ± 0.10	0.07 ± 0.08	0.09 [0.02]	0.05 to 0.14	< **0.001**
Second	0.08 ± 0.08^	0.11 ± 0.09†	0.02 [0.02]	-0.02 to 0.06	0.309
Third	0.04 ± 0.07^	0.08 ± 0.07	0.04 [0.02]	0.00 to 0.07	0.044
Fourth	0.06 ± 0.07^	0.08 ± 0.07	0.03 [0.02]	0.00 to 0.06	0.090

AOK – Combined atropine with orthokeratology; OK – Orthokeratology alone.
CI – Confidence Interval; SD –Standard deviation; SE – Standard error; *P* # – Probability value of unpaired *t*-test for between-group difference, with a Bonferroni-adjusted *P*-value of<0.013 (0.05/4) to indicate significance; ^Significantly different from the first 6-monthly increase in the AOK group (paired t-tests: all $P < 0.001$); † Significantly different from the first 6-monthly increase in the OK group (paired t-tests: $P = 0.003$); Bold – Indicating significance observed; ^†with a Bonferroni-adjusted *P*-value of < 0.008 (0.05/6) to indicate significance.

subjects in the AOK group terminated their treatment (i.e. not suitable to continue the treatment) due to ocular health issues: one had bilateral infiltrative keratitis, one a conjunctival cyst (width < 1 mm), and one bilateral viral conjunctivitis. Three OK subjects were excluded due to bilateral infiltrative keratitis, recurrent bacterial conjunctivitis, despite re-education of personal hygiene, and recurrence of unilateral peripheral corneal erosion after modifications of lens fitting. For these six subjects, lens wear and/or atropine were discontinued immediately at the onset of the ocular symptoms. None of adverse events reported in the current study resulted in a reduction of BCVA or permanent damage to ocular health.

4. Discussion

Both ITT and PP analyses showed an additive effect in retarding axial elongation when 0.01% atropine was used together with *ortho*-k. For subjects who completed the 2-year study, axial elongation was slowed by an additional 0.18 mm in subjects receiving the combined treatment compared with those using *ortho*-k alone over two years (mean ± SD, 0.17 ± 0.19 mm vs 0.35 ± 0.20 mm; adjusted mean [SE], 0.17 [0.03] mm vs 0.34 [0.03] mm, $P < 0.001$). A significant between-group difference in axial elongation was only observed in the first 6-month period, indicating a pronounced additive effect during this period, which accounts for 50% of the overall additive effect. In comparison, Chen et al. failed to observe an additive effect when adding 0.01% atropine after 1-year *ortho*-k [27]. However, in this study, there was highly likely a different baseline mean ± SD axial elongation in children who had chose to add 0.01% atropine to *ortho*-k and those did not (mean ± SD, 0.47 ± 0.15 mm vs 0.41 ± 0.09 mm, $P = 0.04$) [27]. In the current study, further analysis showed that an additive effect was still observed in subgroups of children with low baseline SER (-1.00 to -3.00 D) and those with high baseline SER (over -3.00 D) over two years ($P = 0.011$, 0.012, respectively. Kinoshita et al. reported an additive effect of combining 0.01% atropine and *ortho*-k in children with an initial SER of -1.00 to -3.00 D, but not in those with an initial SER less than -3.00 D [26]. However, no comparison between the current study and Kinoshita's was made in view of the differences in methodologies adopted (e.g. different initial age and SER, and different start point of using 0.01%

Table 4
Summary of symptoms and adverse events reported by subjects received treatment (numbers and percentage) in the two groups.

Symptom	AOK n = 45	OK n = 44	*P*
Photophobia	6(13%)	0	0.006
Halo	4(9%)	2(5%)	0.677
Blurred vision	4(9%)	6(14%)	0.478
Itching	3(7%)	4(9%)	0.671
Dry eye	2(4%)	2(5%)	0.982
Adverse events			
Infiltrative keratitis	1(2%)	1(2%)	0.987
Corneal erosion	1(2%)	1(2%)	0.987
Conjunctival cyst	1(2%)	0	0.320
Conjunctivitis	3(7%)	3(7%)	0.977
Hordeolum	2(4%)	3(7%)	0.627
≥ Grade 2 staining (Efron's scale)	0	1(2%)	0.309
Chicken Pox	1(2%)	0	0.320
Hospitalization	0	1(2%)*	0.309

AOK – Combined atropine with orthokeratology.
OK – Orthokeratology alone.
P – Probability value of comparison of the percentage of subjects between the two groups, using Crosstab analyses.
*Hospitalization due to broken leg for two months.

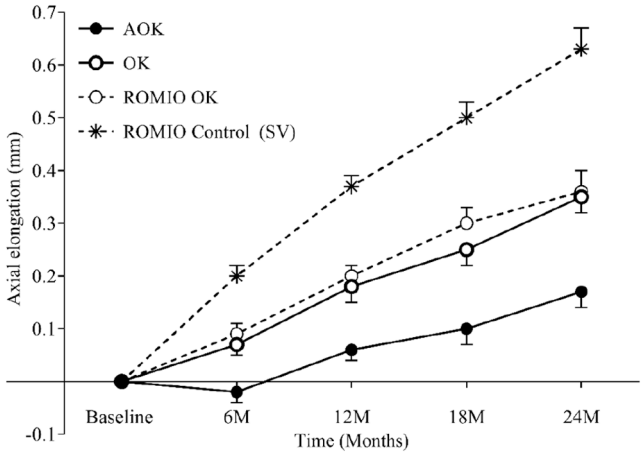

Fig. 2. Cumulative axial elongation over two years in the two groups of subjects in the AOK study compared to subjects in ROMIO study [14].
AOK – Combined atropine with orthokeratology; OK – Orthokeratology alone; ROMIO – Retardation of Myopia in Orthokeratology; SV – Single vision spectacles; Error bars indicate the standard error.

atropine) [26].

Of note, KATT BE Free lenses used in the current study had not been previously evaluated for efficacy in myopia control, and there was no control (single vision glasses) group to determine its efficacy. However, mean ± SD axial elongation in the OK group over two years (0.35 ± 0.20 mm) was not significantly different from that of *ortho*-k subjects in the ROMIO study (0.36 ± 0.24 mm) [14], TO-SEE study (0.31 ± 0.27 mm) [13], or the study by Zhu et al. (0.34 ± 0.29 mm) [16] (all $P > 0.05$). Moreover, the mean ± SD baseline age and myopia in the OK group did not differ significantly from *ortho*-k subjects in the ROMIO or TO-SEE studies (all $P > 0.05$), although OK subjects were younger and had lower SER than *ortho*-k subjects in the study performed by Zhu et al. ($P = 0.026$, < 0.001). These comparisons suggest KATT BE Free lenses is as effective as those *ortho*-k lenses used in previous studies in retarding axial elongation. Baseline age and myopia in the OK group were not significantly different from ROMIO's *ortho*-k subjects and subjects wearing single vision spectacles (i.e. control group) (all $P > 0.05$), and similar protocol was followed in the ROMIO study [14] and the current study. Using the ROMIO study control group as a comparison, axial elongation was slowed by approximately 73% over two years (Fig. 2).

For pupil dilation, the use of a single drop of 0.01% atropine nightly resulted in significant pupil dilation of 0.54 – 0.75 mm and 0.39 – 0.55 mm under photopic and mesopic conditions, respectively, according to CR values for pupil size measurements (0.25 – 0.28 mm) [40]. This mild mydriasis resulting from the use of 0.01% atropine explains why more AOK subjects suffered from photophobia than those in the OK group. Although AOK subjects showed more reduction in accommodation, the changes were not significantly different between the two groups of subjects. Considering the robust baseline accommodation (mean ± SD, AOK vs OK: 13.5 ± 1.9 D vs 12.8 ± 2.2 D, Table 1), a mean reduction of 2.1 D and 1.1 D in the AOK and OK group, respectively, is unlikely to be clinically significant. This study was the first to compare changes in choroidal thickness in children receiving combined 0.01% atropine and *ortho*-k treatment with those wearing *ortho*-k lenses alone over an extended period of two years. There was a greater change in the choroidal thickness (mean: 15.0 – 23.0 μm) in the AOK group than in the OK group (mean: -9.0 – 5.5 μm) at all post-treatment visits. Using a customized software and manual correction, the intra-observer coefficient of repeatability (CR) value for choroidal thickness measurement with Spectralis SD-OCT was reported to be 10.0 μm [35]. Taking this CR into consideration, statistically and clinically significant changes in choroidal thickness were observed in the AOK group, but not in the OK group, at all post-treatment visits.

it is questionable whether the post-treatment changes in choroidal thickness make a major contribution to the changes in axial length in the short term and the long term. After 1-month treatment, an axial shortening was observed in the AOK group (mean ± SD, -0.05 ± 0.05 mm), whilst no significant axial shortening was noted in the OK group (-0.01 ± 0.04 mm), based on a CR value of 0.04 mm for axial length measurements using IOLMaster [41]. Approximately 30% of this axial shortening over one month in the AOK group may be attributable to the thickening of the choroid. Apparently, the shortened axial length over one month was not a direct result of choroidal thickening. Over two years, compared to mean axial elongation of 0.17 mm and 0.35 mm in the AOK and OK group, the between-group difference in choroidal thickness changes (9.5 – 31.5 μm) were negligible. Therefore, it is unlikely that the between-group difference in changes of choroidal thickness had a major contribution to the additive effect of combined treatment in retarding axial elongation over two years. Previous studies reported that baseline age was associated with axial elongation in children who underwent *ortho*-k [14] or low-concentration atropine [38], while the association was not found in the current study. This may be due to different statistical tests used. Linear mixed model was used in the current study, which took into account all relevant baseline parameters.

The mechanism underlying the additive effect resulting from combining 0.01% atropine and *ortho*-k is not clear. It was hypothesized that, by delivering the signal cascade starting at the retina to the sclera, the choroid may play a role in the modulation of axial elongation [42,43]. However, both atropine and *ortho*-k can affect the choroid in children: significant choroidal thickening of 5.5 – 21.0 μm was observed after short-term use (i.e. 1-week, 1-month, or 6-month) of atropine (0.01%, 0.3%, and 1%) [28,44,45]; the choroid was reported to be thickened by approximately 20 μm after 3-week [46] and 12-month [47] *ortho*-k treatment. In the current study, clinically significant thickening in choroid were observed in the AOK group, but not in the OK group. As a greater choroidal thickening were associated with slower axial elongation in the AOK group, it is suggested that the amount of choroidal thickening may play a role in controlling axial eye growth in this group. In addition, in the AOK group, slower axial elongation was associated with a larger increase in the photopic pupil size, while no such association was observed in the OK group. As the enlarged photopic pupil may lead to an increase in the magnitude of ocular higher-order aberrations [48], increased higher-order aberration may provide a directional cue to the retina and consequently alter axial eye growth [49]. Future interventional studies are warranted to clarify the mechanism of the additive

effect of combining atropine and *ortho*-k, thus supporting the use of combined therapy to optimize the treatment effect.

In the current study, to minimize potential bias, measurements of the primary outcome (axial length) were masked, and the same examiner conducted the measurements of each parameter on the same subject for the duration of the study. It was unfortunate that seven parents rejected their child's treatment allocation. However, they did not reveal preference of treatment in the inform and consent process until randomization was performed. One limitation was a high dropout rate (22%) during the study. Of those who dropped out, three subjects lost interest in wearing *ortho*-k lens due to safety concerns about the follow-up examination, and two subjects emigrated abroad. Nevertheless, enough subjects completed the study (a total of 69 subjects completed while 48 were required for completion), to achieve 89% power for the primary analysis, which was higher than the designed threshold of 80%. Subjects who rejected treatment allocation, those lost to follow-up, and those who were no longer suitable to continue the treatment, refused to return for examinations, and most of them had sought *ortho*-k or other myopia control treatment in private practices. Nevertheless, both ITT and PP analyses were performed. Of note, the primary objective is to investigate whether combining 0.01% atropine with *ortho*-k has a better effect in retarding axial elongation, compared with *ortho*-k alone. The lack of control treatment did not compromise the investigation. Moreover, it is unethical to provide control treatment to subjects who are highly likely to have fast myopia progression (i.e. documented myopic progression in SER of at least 0.50 D in the past one year), not to mention the increased chance of dropping out due to inclusion of control treatment. The Hawthorne effect is unlikely, as the primary outcome is axial elongation which was measured by a masked examiner.

In conclusion, an additive effect was observed following combined treatment of 0.01% atropine and *ortho*-k, as axial elongation was slowed by 0.18 mm more with combined treatment of 0.01% atropine and *ortho*-k than with *ortho*-k alone over two years. The combined treatment was well-tolerated, with only a few reversible ocular adverse events and negligible side-effects. The enlarged photopic pupil size and thickened choroid may contribute to the enhanced effectiveness of *ortho*-k and/or atropine for myopia control.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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PC, GPC and VCW designed and planned the study. QT and ALN conducted the experiment. PC analyzed the data and QT wrote the first draft of the paper. All authors interpreted the results and revised the manuscript and approved the final version of the work.

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