Study Protocol and Statistical Analysis Plan

Study Design

This is a randomized, single-blind, controlled trial in which participants will be allocated to the AA group, SAA group, and atropine tapering group in a 1:1:1 ratio. The study protocol strictly follows the Standard Protocol Elements for Intervention Trials (SPIRIT) guidelines[1].

Participants

The hospital's official WeChat account and posters at the outpatient department of Ningbo Eye Hospital are used to recruit suitable pediatric and adolescent participants with myopia. Prior to signing the informed consent form, each participant will be provided with a detailed explanation of the agreement. Participants may withdraw from the study at any time, and personal information will be used solely for medically related research purposes.

Diagnostic criteria

According to the diagnostic criteria for myopia in the Refractive Error Study in Children (RESC) [2], the specific criteria are as follows:

1. SE after cycloplegia < -0.50D;
2. Low myopia > -3.00D;
3. Moderate myopia > -6.00D, ≤ -3.00D;
4. High myopia ≤ -6.00D

Note: The SE after dilation is calculated as the spherical power after dilation plus half the cylindrical power after dilation.

Inclusion criteria

1. Meets the diagnostic criteria for myopia;
2. Age 6–12 years, no gender restrictions;
3. Single or bilateral spherical refractive error between -1.00 and -4.00 D (astigmatism ≤ 1.50 D, anisometropia ≤ 1.50 D);
4. Best-corrected visual acuity in both eyes of 0.20 logMAR or higher;
5. Intraocular pressure (IOP) less than 21 mmHg;
6. Informed consent form signed by a guardian, voluntarily participating.

Exclusion criteria

Patients who meet any of the following criteria will not be eligible to participate in the study:

1. Eye conditions other than refractive errors (e.g., strabismus, amblyopia, keratitis, glaucoma, cataracts, retinal detachment, etc.);
2. Patients with systemic conditions that may affect refractive development (e.g., Down syndrome, Marfan syndrome);
3. Patients with uncontrolled systemic diseases or debilitating conditions, immune deficiencies, or severe primary diseases of the cardiovascular, hepatic, renal, or hematopoietic systems, immune system disorders, or psychiatric conditions;
4. Patients with a history of allergies or hypersensitivity to multiple medications;
5. Patients who have undergone ocular surgery within the past 4 weeks prior to screening;

6. Patients planning to undergo ocular surgery within one year of enrollment;

7. Patients who have participated in other drug clinical trials within the past 3 months prior to screening;

8. Patients who are unable to cooperate with treatment, observation, and assessment.

Elimination criteria

The following criteria will be used to determine whether to exclude patients from the trial:

1. Patients who fail to receive regular intervention treatment;
2. Patients who cannot tolerate rebound reactions and side effects, or who voluntarily withdraw from the study for any other reason;
3. Patients who use other drugs or treatment methods on their own during the trial. The investigator and sponsor will discuss this and assess the impact on safety and efficacy, and ultimately decide on withdrawal;
4. Loss to follow-up of the subject;

During the study, the emergence of other conditions requiring priority treatment.

Termination criteria

The trial will be terminated if any of the following circumstances arise during the trial period:

1. Serious complications or adverse reactions occur during the study, making it inappropriate for the subject to continue participating;
2. Serious adverse events related to the study drug occur;

Subjects who voluntarily withdraw from the clinical study during the study period due to other external reasons.

Sample size

Eligible participants will be randomly assigned to three groups in a 1:1:1 ratio. The G*Power program (version 3) was used to calculate the sample size based on the expected myopia rebound rate at the end of the treatment period for each group

(obtained from our previous pilot trial). With a test power of 1 - beta = 0.9 and a significance level of 0.05, the results indicated that each group requires 50 participants. Assuming a 20% dropout rate, each group would require 60 participants, resulting in a total of 180 participants in the trial.

Randomization and allocation

For grouping, a simple random sampling method will be applied. A set of random numbers will be generated using the random number generator in IBM SPSS Statistics 22.0 software, and participants will be randomly assigned to groups. Based on the grouping results generated by the software, the random allocation cards will be filled out with the following information: serial number, group, and treatment method. The random allocation cards will be placed in sealed envelopes and numbered, with the envelope number matching the serial number of the random allocation card. When participants enter the trial, they will be assigned the corresponding envelope serial number in the order of their entry. Based on the information on the random allocation cards inside the envelopes, participants will be assigned to the specified groups and receive the specified treatments. Participants will be divided into the following three groups: the atropine tapering group, the AA group, and the SAA group. A total of 180 patients will be randomly assigned and distributed to the three treatment groups in a 1:1:1 ratio.

Blinding

Blinded methods will be used to evaluate this study. Due to the unique nature of AA clinical surgery, this study will employ a single-blind method. Efficacy assessments will be conducted by personnel unaware of the group assignments. During the data consolidation phase, blinded statistical analysis will be applied, meaning that statistical analysts will not be aware of the content or group assignments. Only the randomization staff, efficacy evaluators, and data statisticians will be blinded

to ensure that participants, evaluators, and statisticians are unaware of the grouping conditions. However, to implement blinding for recruited participants, an SAA group will be established, where participants are unaware that acupoint stimulation is being performed on sham acupoints.

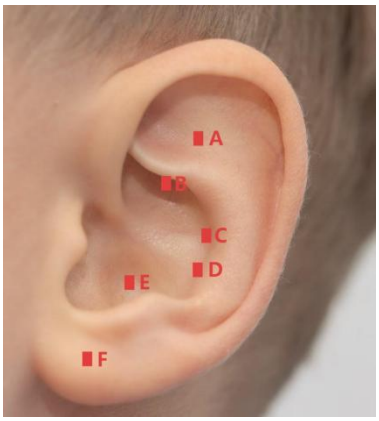
Interventions

Atropine gradual withdrawal group

Patients were administered 0.01% atropine eye drops using a gradual reduction method (Shenyang Xingqi Eye Pharmaceutical Co., Ltd., Shenyang, China, National Medical Products Administration license number: H20243320, 0.04 mg) [27]. The dose was reduced by one day each month, ultimately reducing atropine treatment from seven days per week to complete withdrawal within six months, with a six-month follow-up.

AA group

AA therapy will be added to the gradual reduction method of 0.01% atropine eye drops. As shown in Figure 1. 2; Table 2, ear acupoints: Shenmen (TF4), Heart (CO15), Liver (CO12), Spleen (CO13), Kidney (CO10), and Eye (LO5). The acupuncturist first uses a metal probe to locate the ear acupoints and asks the patient if they feel “deqi,” such as heat, numbness, swelling, or pain. After confirming the ear acupoints, the ears are cleaned with a 75% ethanol solution and dried with sterile, dry cotton balls. Subsequently, the acupuncturist uses the left hand to stabilize the ear while the right hand uses tweezers to apply adhesive tape (10 × 10 mm) containing Wangbuluxing seeds (Wujiang Jiacheng Acupuncture Instrument Co., Ltd., Suzhou, China) to the selected auricular regions.



Group AA applies ear acupuncture points. The red boxes indicate that the acupuncture points are located on the outer surface. The figure shows the distribution of ear acupuncture points, including A [Shenmen (TF4)], B [Kidney (CO10)], C [Liver (CO12)], D [Spleen (CO13)], E [Heart (CO15)], and F [Eye (LO5)].

First, treat the ear acupoint on one side, then leave the tape in place for 5 days. On the 6th day, remove the tape, rest for 2 days, and on the 8th day, apply new tape to the other side of the ear. Changing the tape aims to minimize adverse event (AE) that may result from prolonged stimulation on one side. Additionally, participants will be instructed to self-administer vertical pressure on the Wangbuluxing seeds 15–20 times to achieve sensation, with a duration of 4–5 times daily. The treatment process will last for 18 months, with a follow-up at 6 months.

SAA group

Acupuncturists will use a gradual reduction method with 0.01% atropine eye drops, as used in the control group, and apply skin-colored adhesive tape without Wang Bu Liu Xing seeds to the ear acupoints. During treatment, no massage or acupoint pressure will be applied. The SAA group will follow the same protocol as the AA group for compensatory AA treatment at the end of the study.

Outcome measures

Primary outcomes

Myopia Rebound Rate

The myopia rebound rate is defined as the ratio of the number of individuals experiencing myopia rebound to the total number of individuals in each group. The rebound effect is defined as the rate of myopia progression after discontinuation of treatment exceeding the rate observed during the treatment phase. The rebound effect is assessed based on changes in AL or SE. To enable direct comparison, all data were standardized to an annual rate (mm/y or D/y) by dividing the change in SE or AL by the duration of follow-up (y). Statistical analysis was performed at 3 months and 6 months after dose reduction, and at 1 month, 3 months, and 6 months after discontinuation of medication.

Myopia relapse rate = number of relapses / total number of participants × 100%

SE measurements were taken using a computerized keratometer (KR-1) three times consecutively after ciliary muscle paralysis, with the average data used for analysis. All three readings for the spherical and cylindrical components should differ by no more than 0.25 D. Prior to examining each subject, one drop of compound tropicamide eye drops was administered every 5 minutes. Thirty minutes after the last drop, if the pupillary light reflex is still present or the pupil size is less than 6.0 mm, administer four drops of compound tropicamide eye drops and repeat the examination after 15 minutes. The AL was measured using an ophthalmic biometer (IOLMaster700), with five readings taken and the average value calculated. Measurements were taken at the start of the trial, 3, 6, 9, and 12 months after the intervention, 3 and 6 months after dose reduction, and 1, 3, and 6 months after discontinuation of medication.

Secondary outcomes

（1）Spherical Equivalent Annual growth（SEA）

SE annual growth is defined as the annualized amount of SE after ciliary muscle paralysis. The measurement method is the same as above, with three consecutive measurements taken and the average calculated. Measurements were taken at 3, 6, 9, and 12 months after the intervention, 3 and 6 months after dose reduction, and 1, 3, and 6 months after discontinuation of medication.

SE annual growth = SE change value / follow-up duration (y)

（2）Axial Length Annual growth（ALA ）

The annual growth rate of AL is the annualized value of AL. The measurement method involves taking five consecutive measurements and calculating the average value. Measurements were taken at 3, 6, 9, and 12 months after the intervention, 3 and 6 months after dose reduction, and 1, 3, and 6 months after discontinuation of medication.

Annual growth rate of AL = Change in AL value / Follow-up duration (y)

（3）SE year delay amount

The SE delay is defined as the difference between the annual change in SE in the AA group, SAA group, and atropine withdrawal group after ciliary muscle paralysis. Measurements are taken at 3 months, 6 months, 9 months, and 12 months after intervention, 3 months and 6 months after dose reduction, and 1 month, 3 months, and 6 months after discontinuation of medication.

SE delay = |control group SEA – experimental group SEA|

（4）AL year delay amount

The AL-year delay is defined as the difference between the AL-year change in the AA group, SAA group, and atropine gradual withdrawal group. Measurements are taken at 3 months, 6 months, 9 months, and 12 months after intervention, 3 months

and 6 months after dose reduction, and 1 month, 3 months, and 6 months after discontinuation of medication.

AL-year delay = |control group ALA – experimental group ALA|

（5）SE year delay rate

The SE annual delay rate is defined as the ratio of the SE annual delay amount to the SE annual change amount in the control group multiplied by 100%. Measurements are taken at 6 months, and 12 months after intervention, 6 months after dose reduction, and 6 months after discontinuation of medication.

SE annual delay rate = SE annual delay amount / SE annual change amount in the control group * 100%

（6）AL year delay rate

The AL annual delay rate is defined as the ratio of the AL annual delay amount to the AL annual change amount in the control group multiplied by 100%. Measurements are taken at 6 months, and 12 months after intervention, 6 months after dose reduction, and 6 months after discontinuation of medication.

AL annual delay rate = AL annual delay amount / AL annual change amount in the control group * 100%

（7）ChT

The Sub-Surface Optical Coherence Tomography Angiography (SS-OCTA) system employs Deep Layer™ artificial intelligence for layered measurement of choroidal thickness. Choroidal thickness measurement in the macular region: The macular region of the examined eye is designated as the scanning area, with the fovea centralis as the center, and a radial scan is performed in an ETDRS concentric circle pattern. Choroidal thickness is automatically calculated by the system. In the magnified OCTA image, measurements are taken below the fovea centralis, with nine regions—temporal, nasal, superior, inferior, superior temporal, inferior nasal, inferior

temporal, and superior nasal—within the 0-3mm, 0-6mm, and 0-9mm ranges of the macula. The system automatically calculates the average choroidal thickness across the three ranges. Measurements were taken at the start of the trial, 3, 6, 9, and 12 months after the intervention, 3 and 6 months after dose reduction, and 1, 3, and 6 months after discontinuation of medication.

（8）CVV、CVI

The measurement method for choroidal thickness and choroidal vascular volume uses B-scan mode, with a 9.00 × 9.00 mm vascular OCT scan centered on the fovea of each eye. The system automatically identifies the choroidal vascular structure, reconstructs the choroidal vascular morphology, and quantifies the choroidal vascular volume and vascular index. The vascular index and vascular index within the ETDRS ring range of 0–3 mm, 0–6 mm, and 0–9 mm are measured. Measurements were taken at the start of the trial, 3, 6, 9, and 12 months after the intervention, 3 and 6 months after dose reduction, and 1, 3, and 6 months after discontinuation of medication.

Statistical methods

After collecting the data, it will be loaded into the SPSS-22 program. Using three different methods—histograms, Kolmogorov-Smirnov, and discrete and central indices—we will first determine whether the data distribution is normal. If not, we will apply appropriate transformations. One-way analysis of variance (ANOVA) will be used to compare the myopia rebound rate, annual SE increase, annual AL increase, annual SE delay, annual AL delay, annual SE delay rate, annual AL delay rate, ChT, CVI, and CVV between study groups. If significant results are found, Scheffe post-hoc tests will be conducted. Multiple linear regression analysis will be performed to examine any potential confounding factors. We will examine the assumptions and concerns of the regression model. Additionally, chi-square tests will be used to compare categorical demographic factors within the study group. If variables exhibit abnormal or normal distributions, Kruskal-Wallis tests and ANOVA will be used to compare quantitative variables. If necessary, we will provide post-hoc reports for

these tests. To address missing results in the intention-to-treat (ITT) method, multiple imputation procedures will be used to evaluate the trial. Additionally, 0.05 is considered the significance threshold.

Handling of missing data, withdrawals and subgroup analyses

The ITT method will be used for all analyses, regardless of the number of non-compliant, withdrawn, or lost-to-follow-up cases. We will also conduct analyses according to the protocol. The results of both analyses will be presented.

When there are only a few missing data points, we will use a mixed-effects linear regression method to run routine analyses for each iteration of the multiple imputation process. In the final analysis, fluctuations during the imputation cycle process will be considered. The imputation methods and results for missing data will be displayed.

Subgroup analyses will be conducted based on established prognostic variables. Exploratory subgroup analyses will be performed for the primary outcomes of the following subgroups using 99% confidence intervals (CI) and interaction terms (treatment groups by subgroup): age (6–9 years and 10–12 years at randomization), gender (male, female), and myopia severity (less than -3 D in either eye versus -3 D or higher myopia).

Safety evaluation

Patients will be asked about AEs weekly. Researchers will record AEs in the case report form (CRF), noting the start and recovery dates, severity, relevance to treatment, and how the event was resolved (or not resolved).

At each follow-up visit, patients will be asked about AEs, and intraocular pressure (IOP) and anterior segment photography will be measured. Researchers will record AEs, IOP, and anterior segment photography, including the onset and resolution dates of AEs, severity, their relationship to treatment, and how the event

was resolved (or not resolved) in the CRF. Fundus photography will be performed before and after treatment. AEs associated with AA include itching, swelling, severe pain, wounds, infection, and other discomfort. AEs associated with 0.01% atropine eye drops include photophobia, increased intraocular pressure, eye redness, eye itching, and other discomfort. Patients must report all AEs to the researchers at any time.

Data collection and management

Prior to collecting any data, informed consent forms will be signed by all participants. Throughout the entire study, participants' privacy will be strictly maintained. Investigators are responsible for protecting the privacy of their participants. Participants will be assigned a study identification code, which will protect their privacy. Data and information from participants will be captured on the CRF, and they will remain anonymous throughout the process. Each paper-based data will be checked and added to a password-protected electronic database. To ensure data accuracy, two data entry staff members will use double data entry to input all data into the electronic database. Only approved researchers will have access to the data, thereby ensuring its confidentiality. No information from participants' research will be shared with third parties without their explicit written consent.

Study monitoring and quality control

Prior to recruiting participants, preparatory training will be conducted to ensure that all researchers and relevant personnel adhere to the study protocol and maintain study quality. To ensure consistent outcome assessment and completion of the CRF, all outcome assessors will undergo standardized training simultaneously. Investigators should ensure that all information collected is accurate, complete, and verifiable through source documents. Researchers plan to hold monitoring meetings quarterly to address any issues that may arise throughout the experiment.