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THE EFFECT OF ATROPINE ON THE LENS THICKNESS OF CYNOMOLGUS MONKEYS

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Background: Atropine is an M receptor blocker. It is commonly used clinically to relieve gastrointestinal smooth muscle spasm, inhibit glandular secretion, excite the respiratory center, and relieve the inhibition of the vagus nerve on the heart. In ophthalmology, it is mainly used to relax ciliary smooth muscle, reduce lens adjustment and dilated pupils. Studies in recent years have confirmed that low-concentration (0.01%) atropine eye drops can delay the rapid progress of myopia in children by inhibiting the growth of the eye axis. However, the side effects of improper use of atropine eye drops include dilated pupils, photophobia, blurred vision, and increased intraocular pressure. In China, the use of low-concentration atropine eye drops for myopia in children is still in the clinical trial stage. To this end, researchers in China are working hard to develop atropine drugs to reduce side effects such as dilated ciliary muscles while maintaining the effect of relaxing ciliary muscles. In pre-clinical animal experiments, it is necessary to explore and determine its effective dosage and safety to provide support for long-term clinical applications.

The optical basis of normal vision (emmetropia) is formed when the light (image) entering the eye is accurately focused on the retina through the adjustment of the lens. Excessive adjustment or overly-long eye axis lead to myopia, contrary of which is hyperopia. Based on this principle, if the change in lens thickness before and after drug administration can be measured accurately, we can understand whether the drug has an effect (relaxation or contraction) on the ciliary muscle. Ophthalmology A-type ultrasonic detector is a commonly used diagnostic equipment in ophthalmology. It has in-depth detection ability for ocular tissue. It can detect important physiological indicators, including the depth of the anterior chamber of the eye, the thickness of the lens, the depth of the vitreous cavity, and the length of the axis of the eye, through a one-time operation. It is widely used clinically to measure the refractive power of intra-ocular lenses to be implanted before cataract surgery. Monkeys are the non-human primates closest to humans that have unparalleled similarities with humans in terms of genes, heredity, physiology, anatomy, and pathology. Therefore, monkeys are commonly used animals in ophthalmic drug research.

Purpose: Based on the coupling optical adjustment principle between the ciliary muscle and the lens, use the A-type ultrasonic diagnostic technology to detect the changes in the biological parameters of the eye such as the lens thickness after local administration of different concentrations of atropine eye drops to the eyes of cynomolgus monkeys. Establish the preclinical pharmacodynamic experiment method for evaluating the relaxation effect of new atropine drugs on the ciliary muscle.

Method: 12 healthy juvenile cynomolgus monkeys were randomly divided into 6 groups, two in each group (4 eyes). A single instillation of 30 μ PBS, 0.01%, 0.05%, 0.1%, 0.5%, 1% atropine solution into the conjunctival sac of both eyes. Conduct ophthalmology A-ultrasound examination on both eyes and measure pupil size under anesthesia before drug instillation and 1h, 2h, 6h, 72h and 168h after the instillation.

Result: The average anterior chamber depth, lens thickness, vitreous cavity depth and axial length of the money eye before drug administration were 2.70±0.21, 3. 30±0.08, 12.35±0.18 and 18.36±0.33mm (n=24 eyes), respectively. The pupil diameter of monkey eyes before drug administration was 2.0mm (under 450 lux light intensity). For the PBS group, there were no significant changes in the eye biological indicators at each time point. For the atropine dose groups, the lens thickness of each group showed a dose-related gradual thinning trend at 1h, 2h and 6h after drug administration, and the lens thickness was the thinnest at 6h. The lens thickness showed a high degree or linear correlation (R2=0.9119) with the dosage. 72h and 169h after drug administration, the lens thickness showed doserelated gradual recovery. The depth of the anterior chamber, the depth of the vitreous cavity, and the length of the eye axis in each dose group had no change or slight change unrelated to dose after drug administration. Under constant illuminance, the pupil diameter of each atropine group showed dose-related enlargement (1h, 2h and 6h) and recovery (72h and 168h). Of these, pupil diameter of the 0.05% and 0.01% groups almost or completely recovered 168h after drug administration.

Table 1. The effect of atropine on lens thickness of cynomolgus monkey

Atropine concentration	Before dose	lh	2h	6h	72h	168h (7 d)
NS	100.00±0.00	99.34±1.82	99.55±0.60	99.57±0.92	99.55±0.53	99.65±0.42
1.00%	100.00±0.00	95.29±2.20 [a]	95.34±1.13 [a]	92.57±2.49 [a]	93.05±2.42 [a]	95.83±2.38 [a]
0.50%	100.00±0.00	96.42±1.39	95.65±3.16 [a]	94.89±2.83 [a]	95.97±1.28 [a]	99.07±0.57
0.10%	100.00±0.00	98.01±2.15	96.84±1.49 [a]	96.80±1.08 [ab]	97.04±1.16 [ab]	98.05±1.65
0.05%	100.00±0.00	99.95±0.24 [bc]	99.80±0.70 [bcd]	99.18±0.46 [bcd]	100.13±0.15 [bcd]	100.03±0.37 [b]
0.01%	100.00±0.00	99.30±0.34 [bce]	99.30±0.60 [bc]	99.12±0.22 [bcd]	100.02±0.39 [bcd]	99.85±0.06 [bc]
χ^2	0.000	13.483	16.340	19.183	19.920	12.972
P	1.000	0.019	0.006	0.002	0.001	0.024

Note: To reduce the influence of individual differences between groups before drug administration on the analysis of results, the measured lens thickness of each eye was standardized. Take the actual measured lens thickness (mm) before drug administration for each eye as 100%. Divide the data measured at each time point after drug administration (mm) by the pre-drug data (mm) and then multiply by 100 to obtain the standardized value at each time point after drug administration (%). Compared with PBS group at the same time point, a P<0.05; compared with 1% atropine group at the same time, b P<0.05; compared with 0.5% atropine group at the same time point, c P<0.05; compared with 0.1% atropine group at the same time point, d P<0.05; compared with 0.05% atropine group at the same time point, e P<0.05. (SPSS 13.0 software, Kruskal-Wallis nonparametric test, Mann-Whitney U test).

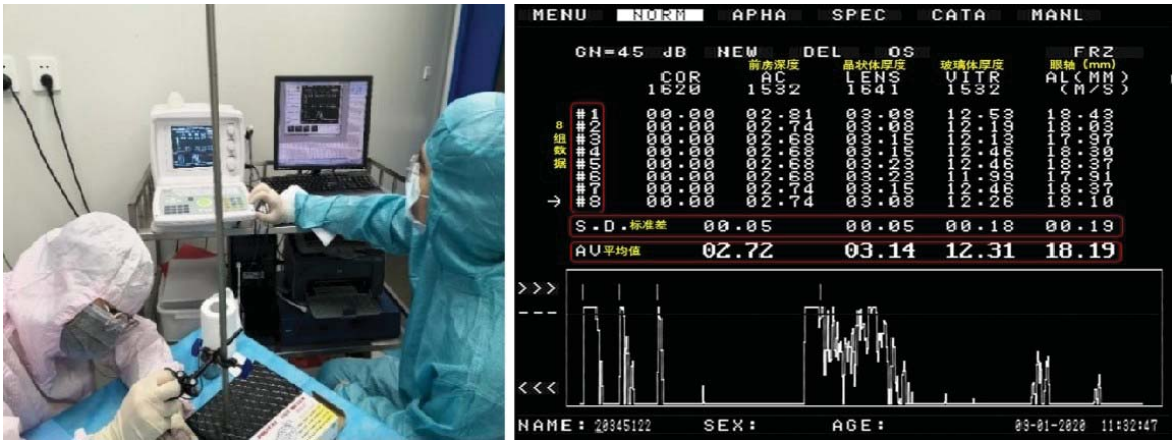

Figure 1. Ophthalmology A-ultrasound inspection site (left) and screenshot of A-ultrasound test results in automatic mode

Table 2 The effect of Atropine on pupil diameter of cynomolgus monkeys (average value ± SD, mm, N = 4 eyes)

Atropine concentration	Before dose	lh	2h	6h	72h	168h (7 d)
0.00%	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0	2.0±0.0
1.00%	2.0±0.0	8.0±0.0 [a]	8.0±0.0 [a]	8.5±0.0 [a]	7.3±0.3 [a]	6.5±0.6 [a]
0.50%	2.0±0.0	8.0±0.0 [a]	8.0±0.0 [a]	8.0±0.0 [aB]	7.0±0.0 [a]	6.0±0.0 [a]
0.10%	2.0±0.0	8.0±0.0 [a]	8.0±0.0 [a]	8.3±0.3 [a]	6.0±1.4 [a]	3.8±0.5 [abc]
0.05%	2.0±0.0	7.0±0.0 [abcd]	7.0±0.0 [abcd]	7.0±0.0 [abcd]	2.0±0.0 [bcd]	2.3±0.3 [bcd]
0.01%	2.0±0.0	6.0±0.0 [abcde]	6.0±0.0 [abcde]	6.8±0.3 [abce]	2.0±0.0 [bcd]	2.0±0.0 [bcd]
χ^2	0.000	23.000	23.000	21.905	21.796	21.792
P	1.000	0.000	0.000	0.001	0.001	0.001

Note: The pupil sizes were the same before drug administration. Pupil sizes were not standardized. Compared with PBS group at the same time point, a P<0.05; compared with 1% atropine group at the same time point, b P<0.05; compared with 0.5% atropine group at the same time point, c P<0.05; compared with 0.1% atropine group at the same time point, d P<0.05; compared with 0.05% atropine group at the same time point, e P<0.05; (Kruskal-Wallis nonparametric test, Mann-Whitney U test).

Conclusion: The dose-effect relationship between atropine and changes in monkey lens thickness was established through A-ultrasound diagnostic technology. And then an in-vivo detection method of the relaxation effect of M receptor antagonists on the ciliary muscle was established, which can be used as an effective preclinical pharmacodynamic evaluation method for the R&D of innovative drugs for the prevention and treatment of myopia in children.