·Basic Research·

Effects of 7-methylxanthine on form-deprivation myopia in pigmented rabbits

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Abstract

• AIM: To determine the effect of 7-methylxanthine (7-MX) on the posterior sclera of form-deprivation myopia (FDM) in pigmented rabbits.

• METHODS: Sixteen pigmented rabbits were monocularly deprived (MD) by suturing the right eyelids after natural eye opening (ten-day old) for a period of 30 days. Two groups of pigmented rabbits were fed either 7-MX (30 mg per kg body weight; n=8) or vehicle control (saline equal volume with 7-MX; n=8). Ocular refractions, axial lengths and body weights were measured at the start and the end of the experiment 30 days later. Electron microscopy was used to measure and determine the collagen fibril diameters in the posterior pole of sclera.

• RESULTS: In vehicle control MD pigmented rabbits, 30 days of MD produced -1.10D± 0.78D of myopia and the axial length increased 0.51mm± 0.09mm. In MD pigmented rabbits fed with 7-MX, 30 days of MD induced only -0.21D± 0.11D of myopia and the axial length increased 0.07mm ± 0.10mm. There was significant change in axial length of vehicle control MD pigmented rabbits (13.11mm ± 0.19mm versus 12.60mm ± 0.06mm; P=0.03). The changes in refraction and axial length of two MD groups' contralateral eyes during the 30 days were not significantly different (2.75D± 0.27D versus 2.75D± 0.35D, P>0.05; 12.60mm ± 0.06mm versus 12.45mm ± 0.14mm, P>0.05). The weights of the two groups pigmented rabbits had no significant changes (187g± 22.1g versus 189g± 19.3g, P>0.05). The diameter of scleral collagen fibers increased in both eyes of 7-MX treated pigmented rabbits. There was significant difference in collagen fibril diameters of inner layer (111.34nm± 28.30nm versus 94.80nm ± 27.52nm, P=0.002) and outer layer (167.92nm ± 55.82nm versus 144.04nm ± 47.02nm, P=0.016) in the posterior sclera between the myopic eyes of vehicle control MD group and contralateral eyes of 7-MX treated MD group.

• CONCLUSION: 7-MX appears to prevent FDM in pigmented rabbits by remodeling the posterior sclera.

• KEYWORDS: 7-methylxanthine; pigmented rabbits; myopia

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INTRODUCTION

Myopia has been a common visual disorder in the young adult population in the world especially in several Asia countries [1-3]. In juveniles of species, such as humans, monkeys, and tree shrews, deprivation of form vision causes excessive axial eye growth and a myopic refractive error [4-6]. Myopia in mammals induced by form-deprivation is accompanied by a decrease in the scleral content of proteoglycans and collagen [7], with reverse changes taking place during recovery [8]. An increased number of small diameter collagen fibrils were found in the longer term in mammalian models of high myopia [9,10].

Chronic treatment of young rabbits with 7-MX increases the content of collagen-related amino acids in posterior sclera and at the same time increases the diameter of the collagen fibrils [11]. Recent work in our laboratory has shown that treatment with 7-MX appears to not only decrease the amount of myopia by around 50% and eliminate the eye elongation induced by form deprivation in guinea pigs, but also to prevent form deprivation myopia-related scleral changes, such as thinning of the sclera and thinning of the

collagen fibril diameter in the posterior sclera[12]. The aim of this study was to determine and verify the effect of systemic 7-MX on the development of form-deprivation myopia and the ultrastructure changes of sclera in pigmented rabbits.

MATERIALS AND METHODS

Materials This study was approved by the Ethics Committee of Sun Yat-sen University in China and complied with the tenets of ARVO statement for the use of Animal in Ophthalmic and Vision Research. Ten-day-old pigmented rabbits were obtained from Animal Experiments Laboratory of Zhongshan Ophthalmic Center (Sun Yat-sen University) and were maternally reared in large cages. The day and night cycle was 12 hours of darkness and 12 hours of white fluorescent lighting. The temperature was maintained at 25℃. Food and water were available ad libitum.

Sixteen pigmented rabbits were randomly divided into two groups. The right eyes of rabbits were monocularly deprived by suturing the right eyelids after natural eye opening (thirteen-day old) for a period of 30 days. One group of pigmented rabbits (n=8) was fed with 7-MX (30mg/kg weight) in every morning for 30 days. The second group of pigmented rabbits (n=8) were fed equal saline in every morning for 30 days. The other eyes served as an internal control group.

Methods

Biological measurements The method of cycloplegia was induced with three drops of tropicamide and refractive errors were measured by means of steak retinoscopy in hand-held, awake animals. Stable refractive errors were generally obtained after 30 minutes when no pupillary response was observed. All refractive errors referred to the spherical-component refractive error, which was defined as the mean refractive error in the horizontal and vertical meridians. The axial length of the eyes was measured by performing ultrasonography with a 10-MHz transducer while the animals were anesthetized with 10% ether in oxygen. The axial length of the eye was defined as the distance from the front of the cornea to the retina. Ocular refraction and axial length were collected at the start and end of the experiment. The body weights of pigmented rabbits also were weighed at the start and the end of the experiment.

Assessment of scleral tissue for electron microscopy After ocular refractive and biometric data had been collected, the animals were administered a lethal dose of pentobarbital sodium (120mg/kg). Eyes were enucleated and residual orbital tissue carefully removed. The eyes were weighed and the horizontal equator, vertical equator were calculated. The cornea was dissected away and the lens was calculated. Ultrathin sections were cut from the posterior tissue punches of the tissue crescents, collected on coated copper grids, and stained for transmission electron microscopy with uranyl acetate, lead citrate, and phosphotungstic acid. Electron micrographs were taken of collagen fibrils in transverse section from the outer (fourth collagen fibril bundle inward from the sclera-episclera boundary), middle (center bundle), and inner layers (fourth layer out from the lamina fusca). Four electron micrographs (21000 magnification) of approximately equal area were taken of each defined scleral layer. Two of these micrographs were obtained from separate areas in one section and two from a later section. Care was taken to ensure that each of these sample micrographs constituted a different collagen fiber bundle. As a result, approximately 400 fibrils were sampled per defined scleral layer, which amounted to approximately 1200 scleral fibrils being sampled from each eye. Fibers were measured with a digitizing tablet, and where fibers were elliptical, the smallest diameter was measured.

Statistical Analysis Statistical analysis was performed using Student's two-tailed *t*-test to compare treated and contralateral eyes. Analysis of variance (ANOVA) and Sheffe F-test were used to compare results in the different groups. The results are reported as means ±standard deviation (SD).

RESULTS

Refraction At the start of the experiment, there were no differences among the three groups in terms of ocular biometric data. In vehicle control MD pigmented rabbits, 30 days of MD produced -1.00D ±0.52D of myopia. But in 7-MX feeding MD pigmented rabbits, 30 days of MD induced an axial myopia of only -0.33D ±0.26D. The refraction of two groups' contralateral eyes had no significant change (2.75D±0.27D versus 2.75D±0.35D, P>0.05).

Axial Length As shown in Table 1, the axial length of vehicle control MD pigmented rabbits was 13.11mm±0.19 mm and increased 0.51 mm ±0.11 mm after 30 days of MD. There was significant change between MD and contralateral eyes in vehicle control group (P=0.03). But in 7-MX feeding MD pigmented rabbits, the axial length was 12.52mm±0.12mm and increased 0.07mm±0.14mm after 30 days of MD. There was no significant change between MD and contralateral eyes in 7-MX group (P>0.05). The axial length of two MD groups' contralateral eyes had no significant change (12.60mm±0.06 mm versus 12.45mm± 0.14mm, P>0.05). There was no significant change in horizontal and vertical equator (P>0.05).

Weight As shown in Table 2, the weight of 7-MX feeding MD and saline feeding MD pigmented rabbits were 187g± 22.1g versus 189g±19.3g after 30 days of MD. There were no significant change in body weight between the two groups (P>0.05). There was no significant changes in the weight of eyeballs and lens between the two groups (P>0.05).

Table 1 Axial length, horizontal and vertical equator of two groups pigmented rabbits (mm)

Groups	7-MX		Saline	
	right	left	right	left
Axial Length	12.52±0.12	12.45±0.14	13.11±0.19*	12.60±0.06
Horizontal Equator	13.09±0.21	13.25±0.24	13.38±0.12	13.33±0.10
Vertical Equator	14.55±0.29	14.37±0.21	14.73±0.23	14.74±0.15

Compared with contralateral eyes: $P<0.05$*, $P<0.01$**.

Table 2 The weight of eyes, lens and bodies in two groups of pigmented rabbits g

Groups	7-MX		Saline	
	right	left	right	left
eyes	1.30±0.11	1.30±0.09	1.29±0.05	1.28±0.04
lens	0.15±0.01	0.15±0.01	0.16±0.01	0.16±0.01
bodies	187±22.1		189±19.3	

Scleral Collagen Fibril Distribution The collagen fibril diameter increased in the direction of the inner-outer sclera in pigmented rabbits eyes (Figure 1). After 30 days of MD, the collagen fibril diameter decreased in the three posterior scleral layers (inner layer: 94.80nm ±27.52nm versus 104.22nm ±29.56nm; middle layer:132.56nm ±44.96nm versus 145.28nm ±40.30nm; outer layer: 144.04nm ±47.02nm versus 153.88nm±47.46nm) in MD eyes compared with the contralateral eyes in vehicle control group. There was no significant difference were seen in all three layers of the sclera in vehicle group (P=0.07, inner layer; P=0.14, middle layer; P=0.32, outer layer).

In the 7-MX treated MD group, compared with the vehicle control MD animals, the collagen fibril diameter in sclera from 7-MX treated animals was increased not only in the MD eyes, but also in the contralateral eyes. In fact, there was no significant difference seen in all three layers of the posterior sclera between MD eyes and contralateral eyes in 7-MX treated MD group (P>0.05). A significant difference was apparently seen in collagen fibril diameters of inner layer (111.34nm±28.30nm versus 94.80nm±27.52nm, P=0.002) and outer layer (167.92nm ±55.82nm versus 144.04nm ±47.02nm, P=0.016) in the posterior sclera between the myopic eyes of vehicle control MD group and contralateral eyes of 7-MX treated MD group.

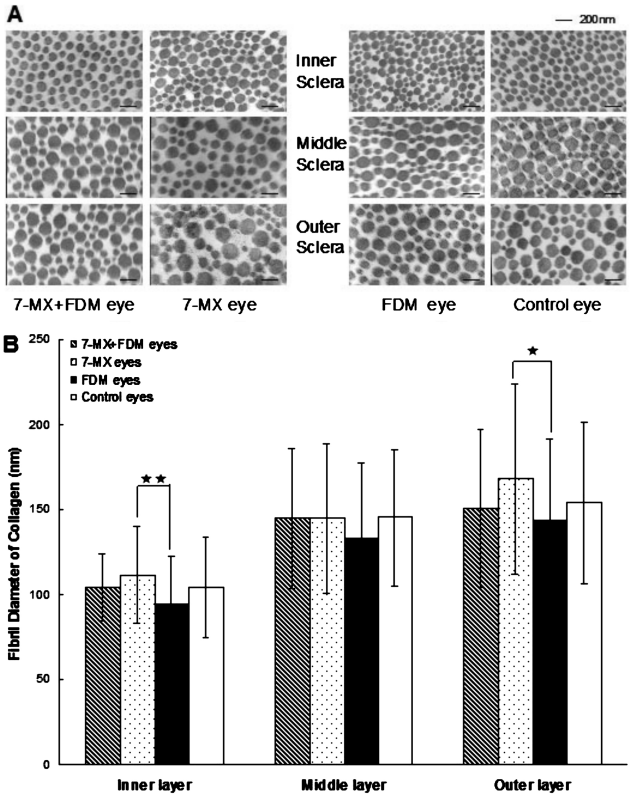

Figure 1 Electron micrographs and scleral collagen fibril distribution at the posterior sclera of the vehicle control and 7 -MX treated pigmented rabbits. A: Electron micrographs showing a transverse section through scleral collagen fibrils, at the posterior pole, from each layer of the vehicle control and 7-MX treated pigmented rabbits; B: Scleral collagen fibril distribution at the posterior pole of the MD and contralateral eyes of the vehicle control and 7-MX treated pigmented rabbits. The fibrils were counted in three separated areas and the distribution of different fibril diameters in the total number of fibrils was determined. A significant difference was seen in collagen fibril diameters of inner layer (P=0.002) and outer layer (P=0.016) in the posterior sclera between the myopic eyes of vehicle control MD group and contralateral eyes of 7-MX treated MD group. ★ means P<0.05, ★★ means P<0.01.

DISCUSSION

The results demonstrated that 7-MX was effective in reducing FDM of pigmented rabbits. These findings add further support to a role for 7-MX slows down axial elongation in myopic kids[13] and guinea pigs[12].

7-MX was a nonselective antagonist of adenosine receptor. Adenosine receptors have four subtypes, including A1, A2A, A2B and A3. All the four subtypes have been found localized in sclera, choroid, retinal pigment epithelium and retina from guinea pigs [14]. Adenosine receptor may play a role in FDM in guinea pigs. Myopia induced by form deprivation is known to be accompanied by changes in the activity of various retinal neurotransmitters [15-19], and several drugs that interfere with neurotransmission appear to modulate the development of experimental myopia [15, 20-23].

Particular interest has been focused on acetylcholine and dopamine in relation to deprivation myopia. Both of these neurotransmitters are modulated by adenosine receptors. Adenosine has a general homeostatic role in the regulation of cell metabolism, but also a specific neuromodulatory role with A1 receptors having an inhibitory role and A2 receptors having a facilitatory role in the release of neurotransmitters such as dopamine, noradrenalin, acetylcholine, glutamate and serotonin [24]. In the CNS, adenosine A2A receptors are known to be co-localised with and interact with dopamine D2 receptors. Thus, inhibition of A2A receptors enhances postsynaptic dopamine D2 receptor transmission [25], dopamine D2 activation antagonizes tonic activation of adenosine A2A receptors, and blockade of dopamine D2 receptors unmasks strong adenosine A2A receptor activation[26]. The content of dopamine is reduced in retina from visually deprived tree shrews [27], and administration of dopaminergic substances seems to inhibit the development of experimental myopia in mammals [18, 20]. The ability of dopamine to neutralize experimental myopia in chickens may be linked to an effect on D2 receptors[21]. A possible role for the retinal pigment epithelium in the control of ocular growth has been implicated on the basis of in vitro experiment and experimental myopia in chicks [28]. Adenosine is believed to play a role in the response of the retinal pigment epithelium to light[29].

Topically applied muscarine acetylcholine antagonists, like the non-selective antagonist atropine and the M1 selective antagonist pirenzepine have been shown to reduce myopia progression and axial eye growth in humans [30-32], and in animals during form deprivation [33,34]. The mechanism of action of atropine and pirenzepine has not been established, but muscarinic M1 to M5 receptors have been identified in guinea pig retina, choroid, and sclera, and expression of M1 and M4 receptors in posterior sclera was increased in form deprivation myopia [35]. Interestingly, in other cell systems, muscarinic and adenosine receptors interact. It is therefore conceivable that adenosine and muscarinic acethylcholine receptors in the eye interact, and that blocking of adenosine receptors blocks form deprivation myopia due to modulation of muscarinic acetycholine transmission, or vice versa. It is likewise conceivable that simultaneous blocking of both adenosine and muscarinic acethylcholine receptors could have an additive effect on eye elongation during form deprivation.

In conclusion, treatment with 7-MX appears not only to decrease myopia and eye elongation induced by form-deprivation in pigmented rabbits, but also to prevent the FDM-related reduction of collagen fibril diameter. It is probable that the effect is related to blocking of ADOR, but which specific subtype is involved, and whether the effect is directly in the sclera, or through the retina, the choroid or the retinal pigment epithelium remains to be investigated.

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