·Basic Research·

Mechanism of the DL-alpha-aminoadipic acid inhibitory effect on form-deprived myopia in guinea pig

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Abstract

• **AIM: To investigate the effect of intravitreal injection of DL-alpha-aminoadipic acid (DL-α-AAA) on ocular refractive state and retinal dopamine, transforming growth factor-β_2 (TGFβ_2), vasoactive intestinal polypeptide (VIP) in guinea pig form-deprived myopia.**

• **METHODS: Four-week-old pigmented guinea pigs were randomly assigned to 4 groups: normal control, deprivation, deprivation plus DL-α-AAA, deprivation plus saline. Form deprivation was induced with the self-made translucent eye shields, and lasted for 14 days. 8μg DL-α-AAA was injected into the vitreous chamber of deprived eyes. The corneal radius of curvature, refraction and axial length were measured. Retinal dopamine content was evaluated by the high-performance liquid chromatography with electrochemical detection, and TGFβ_2 and VIP protein were detected by Western blotting.**

• **RESULTS: Fourteen days of eye occlusion caused the axial length to elongate and become myopic in the form-deprived eyes, with the decrease of retinal dopamine and the increase of TGFβ_2 and vasoactive intestinal polypeptide (VIP) protein. Intravitreal injection of DL-α-AAA could inhibit the myopic shift from (-3.65±1.06)D to (-1.48±0.63)D, P<0.01 due to goggles occluding and cause the decrease of retinal TGFβ_2 protein in the deprived eyes. However, intravitreal injection of DL-α-AAA had no significant effect on retinal dopamine and VIP protein in deprived eyes. Retinal TGFβ_2 protein correlated highly with the ocular refraction (y=-3.34+0.31/x, F=74.75, P<0.001) and axial length (y=8.39-0.02/x, F=48.32, P<0.001) in different treatment groups.**

• **CONCLUSION: Intravitreal injection of DL-α-AAA is effectively able to suppress the development of form deprivation myopia, which may be associated with retinal TGFβ_2 protein in guinea pigs.**

• **KEYWORDS:** DL-alpha-aminoadipic acid; form-deprivation myopia; TGFβ_2; Müller cell; retina
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INTRODUCTION

Alpha-aminoadipic acid (α-AAA) is a six-carbon chemical analog of the excitatory amino acid, L-glutamic acid. It is well known that D-, L- and DL-isomers have gliotoxic effects in the retina both *in vitro*[1] and *in vivo*[2]. With regard to these isomers, D-α-AAA and DL-α-AAA individually exhibit selective damage to retinal Müller cells; however, L-isomer is found to cause additional neuronal injury. In our previous study, it has been shown that the intravitreal DL-α-AAA specifically acts on retinal Müller cells, and effectively ameliorates the development of form-deprived myopia in guinea pig [3]. Subsequently, we revealed that retinal Müller cells in the guinea pig myopic eye can synthesize dopamine, transforming growth factor-β_2 (TGFβ_2), vasoactive intestinal polypeptide (VIP) [4,5]. But it is unclear whether the DL-α-AAA inhibition on myopia is associated with these retinal factors. In the present study, we investigate the association between DL-α-AAA inhibitory effect on myopia and retinal dopamine, TGFβ_2 and VIP.

MATERIALS AND METHODS

Materials Healthy triad color guinea pigs (clean grade, n=48, aged 4 weeks, weighed 180-220g) were obtained from the Animal Center of Xiangya Medical College, and were randomly divided into four groups: normal control, deprivation, deprivation plus DL-α-AAA, deprivation plus saline. All animal experiments were performed in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No.86-23, revised 1986). Approval for the project was obtained from the Animal Ethics Committees of Xiangya Medical College.

Guinea pigs were intraperitoneally injected with 30mg/kg sodium pentobarbital. Then, the self-made translucent eye shields were sutured to the tissue surrounding the right eye socket on the right eye. It was observed that occluders did not

Table 1 Effect of DL-α-AAA on refraction, axial length and corneal radius of curvature (n=12)

Groups	Corneal curvature(mm)	Refraction (D)	Axial length (mm)
Normal control			
Right	3.57±0.03	+0.80±0.25	8.16±0.05
Left	3.58±0.02	+0.83±0.25	8.15±0.04
Deprivation			
Right	3.59±0.02	-3.65±1.06^{b,d,f}	8.41±0.09^{b,d,f}
Left	3.57±0.03	+0.86±0.20	8.17±0.06
Deprivation plus DL-α-AAA			
Right	3.58±0.04	-1.48±0.63^{b,d}	8.30±0.06^{b,d}
Left	3.58±0.01	+0.86±0.22	8.15±0.05
Deprivation plus saline			
Right	3.57±0.04	-3.55±1.12^{b,d,f}	8.40±0.08^{b,d,f}
Left	3.59±0.03	+0.84±0.25	8.16±0.06

Data are expressed as the mean±SD; ^{b}P<0.01 *vs* the left eye; ^{d}P<0.01 *vs* the normal control group; ^{f}P<0.01 *vs* the deprivation plus DL-α-AAA group.

compromise the cornea, and form-deprived eyes could freely blink behind occluders. Animals were then reared under 12-12 hours light/darkness cycle.

Fourteen days after occlusion, the occluders were removed. Its corneal radius of curvature was measured with a keratometer (Topcon, OM-4, Japan), and refraction was measured with a streak retinoscope. Type-A ultrasonic examination (Cinescan A/B, Quantel Medical, French) was conducted to determine the axial length. Each animal was measured three times and the mean value was used for the following analysis.

Methods DL-α-AAA (Sigma, St. Louis, MO, USA) was freshly prepared by dissolving into physiological saline solution. At the first day and the seventh day of occlusion, guinea pigs in the deprivation plus DL-α-AAA group were intravitreally injected with 5μL DL-α-AAA solution contained 8μg DL-α-AAA using a 25μL Hamilton syringe. As a control, physiological saline solution (vehicle) was injected in the same way. In the current study, the dosage of DL-α-AAA was chosen on the basis of previous work in guinea pigs[3].

Retinal dopamine content was detected by the high-performance liquid chromatography (HPLC) [4] and was plotted as nanogram dopamine per milligram retina.

Retinal samples for western blotting detection were frozen in liquid nitrogen. The blot was probed with a polyclonal antibody against rabbit TGFβ$_2$ and VIP (Santa Cruz, USA), working concentration 1:500. Target strap is performed grey value analysis by Bandscan 5.0 image analysis software, GAPDH as the internal control; the relative expression of target protein is calculated.

Statistical Analysis All data was expressed as mean±SD, and analyzed with SPSS11.5 software. One-way ANOVA was used to analyze ocular refraction, axial length, corneal radius of curvature, and the content of retinal DA, TGFβ$_2$, and VIP. The correlation in axial length, refraction and retinal TGFβ$_2$ was analyzed by the regression of an inverse function.

RESULTS

Effect of DL −α −AAA on Ocular Refractive State Fourteen days after occlusion, the deprived eyes became myopic with a significant increase in its axial length, which showed statistically significant difference when compared with its fellow control eyes and age-matched normal control eyes (P <0.01). Intravitreal injection of DL-α-AAA significantly reduced the degree of myopia (from -3.65±1.06D to -1.48±0.63D, P<0.01) and retarded the increase of axial length (from 8.41±0.09mm to 8.30±0.06mm, P<0.01) in the deprived eyes. However, the myopia development was not suppressed completely by 8μg DL-α-AAA treatment, because its refraction showed statistically significant difference when compared with its fellow control eyes (P<0.01) and normal control eyes (P<0.01). In contrast, intravitreal injection of saline (vehicle) caused no statistically significant effect on the refraction in the deprived eyes. No statistically significant difference was observed in corneal radius of curvature in different treatment groups (Table 1).

Effect of DL−α−AAA on Retinal Dopamine, TGFβ$_2$ and VIP Fourteen days of form-deprivation induced a significant decrease in retinal dopamine content of the deprived eyes when compared with fellow control eyes (P <0.01) and age-matched normal control eyes (P<0.01). However, 14 days of form-deprivation induced a significant increase in retinal TGFβ$_2$ and VIP protein (P<0.01). Intravitreal injection of DL-α-AAA significantly caused the decrease of retinal TGFβ$_2$ protein in the deprived eyes, when compared with those in the deprivation group (P <0.01). There was no statistically significant difference in retinal dopamine content and VIP protein of the deprived eyes between the deprivation group and the deprivation plus DL-α-AAA group. In contrast, intravitreal injection of saline (vehicle) caused no statistically

Table 2 Effect of DL-α-AAA on retinal dopamine, TGFβ$_2$ and VIP (n=12)

Groups	Dopamine(ng)	TGFβ$_2$	VIP
Normal control	1.52±0.57	0.09±0.03	0.14±0.05
Deprivation	0.65±0.15^b	0.69±0.13^{b,d}	0.47±0.09^b
Deprivation plus DL-α-AAA	0.70±0.17^b	0.43±0.10^{b,d}	0.45±0.11^b
Deprivation plus saline	0.67±0.18^b	0.71±0.15^{b,d}	0.49±0.15^b

Data are expressed as the means ± SEM; ^bP<0.01 *vs* the normal control; ^dP<0.01 *vs* the deprivation plus DL-α-AAA group.

significant effect on retinal dopamine content, TGFβ$_2$ and VIP protein in the deprived eyes (Table 2，Figure 1).

The ocular refraction correlated highly with retinal TGFβ$_2$ protein in different treatment groups (y=-3.34+0.31/x, R^2=0.62, DF=46, F=74.75, P<0.001). The axial length and retinal TGFβ$_2$ protein were also correlated in different treatment groups (y=8.39-0.02/x, R^2=0.51, DF=46, F=48.32, P<0.001) (Figure 2).

DISCUSSION

Myopia is a visual disorder affecting about one half of the world's population. It is also a socio-economic-health problem of considerable proportions. Its prevalence shows a rising tendency, especially in Asian countries such as China [6,7], Japan [8,9] and Singapore [10,11]. However, there is no generally accepted and approved method or drug for preventing myopia.

Müller cells are the predominant class of glial cells in the vertebrate retina. They span the entire width of retina from the inner to the outer limitant membrance, and its processes surround most of retinal neurons. Together with retinal neurons, they constitute a "neuron-glia" regulatory network that is involved in the retinal functional activities, including supporting and nourishing neurons, maintaining the stability of extracellular ion concentration, and participating in glutamate cycle and synaptic signal transduction, *etc* [12]. It has been shown that Müller cells may participate in the formation of myopia in the guinea pig, and it is a significant source for signaling factors of TGFβ$_2$ in the retina [3,5].

Seko *et al* [13] has reported that the content of TGFβ$_2$ is increased in the posterior pole of retina-retinal pigment epithelium-choroid complex and sclera of the deprived eyes in chicken, suggesting that TGFβ$_2$ participate in the development of myopia. However, Honda *et al* [14] has reported that TGFβ$_2$ protein are reduced in retina of the deprived eyes in chicken. In addition, Jobling *et al* [15] has found that form deprivation in the early stage has no significant effect on TGFβ$_2$ content in retina, and TGFβ$_2$ content begins to decrease on day five in the tree shrew. In our study, form deprivation induced a significant increase in retinal TGFβ$_2$ protein of the deprived eyes in the guinea pig. Together these results suggest that there are the controversial results of retinal TGFβ$_2$ in the form deprivation myopia, which may be associated with the species differences. So, the exact relation between retinal TGFβ$_2$ and myopia remains to be verified by further studies.

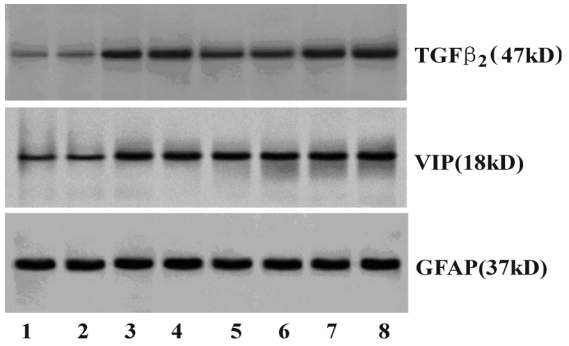

Figure 1 Western blotting technique detection of retinal TGFβ$_2$ and VIP protein in the guinea pig 1,2: normal control; 3,4: deprivation; 5,6: deprivation plus DL-α-AAA; 7,8: deprivation plus saline.

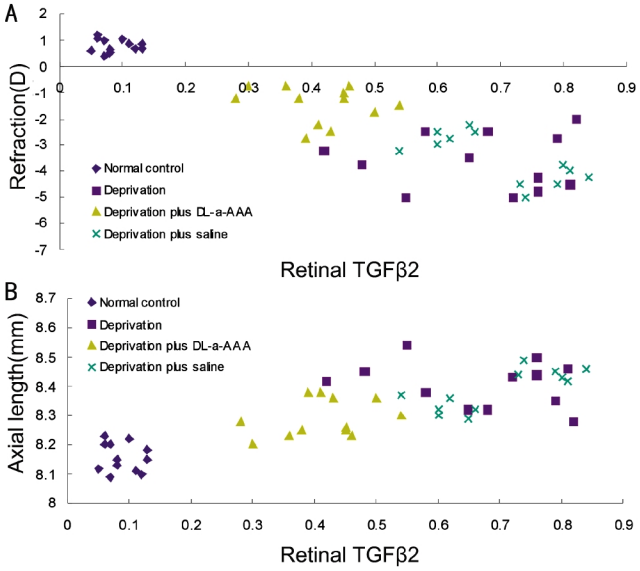

Figure 2 A: Relation between the ocular refraction and retinal TGFβ$_2$ protein in different treatment groups (y=-3.34+0.31/x, F=74.75, P<0.001); B: Relation between the axial length and retinal TGFβ$_2$ protein in different treatment groups (y=8.39-0.02/x, F=48.32, P<0.001).

DL-α-AAA has gliotoxic effects in the retina and exhibits selective damage to retinal müller cells [16]. In the present study, we found that intravitreal injection of DL-α-AAA significantly reduced the degree of myopia and retinal TGFβ$_2$ protein in the deprived eyes, and retinal TGFβ$_2$ protein correlated highly with the ocular refraction and axial length. These evidences indicate that retinal TGFβ$_2$ protein may participate in the inhibitory effect of DL-α-AAA on myopia in guinea pig. Although retinal Müller cells is also a significant source for retinal dopamine and VIP, intravitreal

injection of DL-α-AAA caused no significant change of retinal dopamine and VIP in the guinea pig myopic eyes.

In summary, our data showed that intravitreal injection of DL-α-AAA could effectively retard the myopic development in the form-deprived guinea pig eyes, which may be associated with the change of retinal TGFβ$_2$ protein.

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