Original Article

Exogenous Levodopa Increases the Neuro-retinal Dopamine of Guinea Pig Myopic Eyes *in vitro*

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Abstract

Purpose: In our previous work, it has been shown that intraperitoneal injection of L-DOPA can inhibit the development of occlusion myopia in guinea pigs, and increase levels of retinal dopamine. The aim of this study was to investigate whether exogenous L-DOPA can be converted into dopamine in cultured retina of guinea pig eyes subjected to visual deprivation, and to evaluate whether müller cells are involved in the processing of retinal dopamine induced by L-DOPA.

Methods: Fifty-eight guinea pigs were randomly divided into 2 groups at the age of 4 weeks: normal control and visual deprivation. Form deprivation was induced with translucent eye shields over the right eye, and lasted for ten days. Corneal curvature, refraction and axial length were measured in all animals. In vitro, neuro-retina and müller cell were cultured, and L-DOPA was added to the culture medium at three concentrations: 1 μM, 10 μM and 100 μM. Subsequently, dopamine content was evaluated by high-performance liquid chromatography, and apoptotic cells were identified by TUNEL staining.

Results: Ten days of occlusion caused the affected eyes to elongate and become myopic in guinea pigs. Compared with the deprivation group, 10 μM L-DOPA treament significantly raised dopamine content in cultured retina and müller cells ($P<0.05$). However, 1 μM and 100 μM L-DOPA treatment caused no increase in dopamine levels ($P>0.05$). Apoptotic nuclei were detected in the ganglion cell layer (92.5%±8.3%) and inner nuclear layer (46.8%±9.1%)of cultured retina treated with 100 μM L-DOPA. Moreover, 100 μM L-DOPA also caused apoptosis of retinal müller cells, at a mean rate of 59.4 ±11.3%.

Conclusion: Our results suggest that exogenous L-DOPA can cause an increase in retinal dopamine in form-deprived guinea pig eyes in vitro, and that müller cells are involved in the increase in retinal dopamine. (***Eye Science 2011; 26:211-216***)

Keywords: levodopa; organotypic culture; dopamine; müller cell; form deprivation myopia

Myopia is a visual disorder affecting about one-half of the world's population. Its incidence in school-age children is increasing markedly in many parts of the world, especially in Asian countries such as China[1,2], Japan[3] and Singapore[4,5]. Therefore, a drug treatment that can either prevent myopia or limit its progression is urgently needed.

Dopamine is one of the major neurotransmitters in the retina, and is involved in the signal transmission in the development of experimental myopia. Visual deprivation leads to ocular enlargement and myopic refractive error, and also reduces the retinal concentration of dopamine and its metabolites[6,7,8,9]. Changes in chicken retinal dopamine content displayed a circadian rhythm, showing a downtrend during the night, and form deprivation mainly led to a decline in retinal dopamine content during the day[10,11]. By the same token, the level of retinal dopamine and its metabolite was also reduced in lens-induced myopic chick eyes[12].

In our previous study, it was shown that repeated intraperitoneal injection of levodopa (L-DOPA) could inhibit the myopic shift owing to occluding goggles and compensate retinal dopamine in guinea pigs[13]. In the present study, we used the established guinea-pig myopia to investigate the effect of exogenous L-DOPA on retinal dopamine in organotypic culture. It has been shown that retinal Müller cells can synthesize dopamine[14,15], which is closely related to myopic formation. Therefore, it is necessary to

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evaluate whether the retinal Müller cell is involved in the increase of retinal dopamine caused by exogenous L-DOPA in the study.

Materials and methods

Induction of form-deprived myopia

Four-week-old pigmented guinea pigs were obtained from the animal center of Xiangya Medical College. All 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 in 1986). Approval for the project was obtained from the Animal Ethics Committees of Xiangya Medical College. The specific methods inducing form-deprived myopia refer to a previous study[13]. Tens days after occlusion, the radius of corneal curvature was measured with a keratometer (Topcon, OM-4, Japan), and ocular refraction was measured with a streak retinoscope. Type-A ultrasonic examination (Cinescan A/B, Quantel Medical, French) was conducted to determine the axial length. The ultrasound frequency was 11 MHz. In the study, the term "axial length" refers to the distance from the corneal apex to the vitreoretinal interface. Each animal was measured three times and the mean value was used for the following analysis.

Retinal culture and exogenous L-DOPA treatment in the deprived eyes

L-DOPA (Sigma, St. Louis, MO, USA) solution (10 mg/ml) was freshly prepared by dissolving into physiological saline solution. After myopia was induced, guinea pigs were randomly divided into four groups:1)Deprivation;2)Deprived plus 1 μM L-DOPA;3)Deprived plus 10 μM L-DOPA;4)Deprived plus 100 μM L-DOPA.

After enucleation,the anterior segments were cut off, leaving eye cups. Then, the neural retinae were teased off the sclera using a fine brush, and cut into a piece of 10×10 mm around the pecten using a surgical blade. The retinae were placed on a sterile, perforated, high-grade stainless steel grid support bench placed in a six-well plate. The culture medium consisted of MEM, supplemented with 2.8 ml glu tamine(Gibco Brl, Eggenstein, Germany); 10% newborn calf serum (Gibco Brl, Eggenstein, Germany); 673U/ml penicillin; and 673 μg/mL streptomycin. A brush was used to orient the retinae with the ganglion cell side facing upward.

After 12 hours of organotypic culture, L-DOPA was added to the culture medium at three final medium concentrations:1 μM, 10 μM and 100 μM. After 24 hours of L-DOPA treatment, retinal dopamine content was detected by the high-performance liquid chromatography-electrochemical detection (HPLC-EC)(n=6).

Primary culture of retinal Müller cells and exogenous L-DOPA treatment

The specific cultural methods refer to a literature[13]. Cells were identified by immunocytochemical analysis with antibodies against the Müller-cell markers vimentin protein (Santa Cruz,CA,USA). When the cell density was 5×10^4 cm^{-2} in the second generation, L-DOPA solution (10 mg/ml) was added into Müller cells for three hours. The concentration of L-DOPA is 1 μM, 10 μM and 100 μM, separately. Müller cells are divided into four groups: 1) Deprivation; 2) Deprived plus 1 μM L-DOPA; 3)Deprived plus 10 μM L-DOPA; 4) Deprived plus 100 μM L-DOPA.

Dopamine content detected by HPLC-EC

The specific methods refer to a literature[13].Dopamine content is expressed with ng/mg in neural retinae, and ng/ml in Müller-cell culture fluid.

In situ detection of apoptosis in the cultured retinae by TUNEL staining

After L-DOPA treatment, the cultured retinae and Müller cells(n=2)were fixed by neutral buffered formalin for further TUNEL staining. DNA nick end-labeling was performed using an apoptosis-detection kit (Boeheringer Mannheim GmbH, Mannheim, Germany). The number of apoptotic cells was expressed as the ratio of TUNEL-labeled nuclei to the total number of nuclei. Cells were counted in three fields in six micrographs at a magnification of 40×10.

Statistical analysis

Values are expressed as means±SEM. The corneal curvature, refraction and axial length were analyzed by the t-test in guinea pigs. Dopamine content was analyzed by the one-way ANOVA. Probability values less than 0.05 were accepted as statistically significant.

Results

The effect of occlusion on the refractive state and axial length in guinea pigs

After the right eyes were occluded for ten days in guinea pigs, the deprived eyes became myopic(–3.50± 1.13D), which showed a statistically significant difference when compared with the fellow control eyes and age-matched normal control eyes (P<0.01). Ten days of form-deprivation induced a significant increase in axial length of the deprived eyes (8.40± 0.11mm), when compared with the fellow control eyes and age-matched normal control eyes (P<0.01). However, There was no statistically significant difference in radius of corneal curvature in different groups(P>0.05) (Table 1).

Table 1 Difference of corneal curvature, ocular refraction and axial length in guinea pigs ($\bar{x}\pm s$)

Groups	Eyes	n	Corneal curvature(mm)	Refraction(D)	Axial length(mm)
Normal control	Right	10	3.57±0.05	+0.65±0.30	8.25±0.08
	Left	10	3.58±0.04	+0.50±0.25	8.26±0.08
Deprivation	Right	48	3.58±0.07	–3.50±1.13#*	8.40±0.11#*
	Left	48	3.59±0.04	+0.50±0.32	8.27±0.07
t			0.533	21.993	5.022
P			>0.5	<0.001	<0.001

P<0.001 vs normal control; *P<0.001 vs the left eye

Table 2 Difference of dopamine content in the deprived eyes caused by L-DOPA treatment *in vitro* ($\bar{x}\pm s$, n=6)

Groups	Deprivation	1 mM L–DOPA	10 μM L–DOPA	100 μM L–DOPA	F	P
Cultured retina (ng/mg)	0.58±0.13*	0.67±0.14*	0.94±0.21	0.65±0.16*	3.682	<0.05
Müller cell(ng/ml)	0.15±0.05*	0.17±0.06*	0.29±0.08	0.12±0.06*	4.021	<0.05

P<0.05 vs Deprived plus 10 μM L–DOPA

The effect of L-DOPA on dopamine content in the cultured retinae and Müller cells of the deprived eyes

In the cultured retinae, dopamine content was (0.58±0.13) ng/mg. There was no statistically significant difference in dopamine content per milligram of the retinae between the deprivation group and the deprived plus 1 μM L-DOPA group (P> 0.05). Compared with the deprivation group, 10цM L-DOPA treatment significantly raised the retinal dopamine content (P<0.05). However, 100 μM L-DOPA treatment caused no statistically significant effect on retinal dopamine content (P>0.05).

Retinal Müller cells grew and adhered to the wall of a flask, with large applanate cell bodies, over 95% culture cells showed positive expression of vimentin (Figure 1). By the same token, 10 μM L-DOPA treatment also significantly raised the retinal dopamine content in the cultured retinal Müller cells (P <0.05). However, 1 μM and 100 μM L-DOPA treatment caused no statistically significant effect on retinal dopamine content (P>0.05).

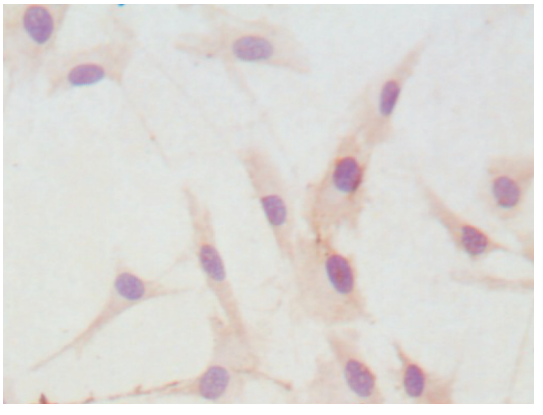

Figure 1 Positive expression of vimentin in retinal Müller cells in the deprived eyes ×400

The toxicity of L-DOPA on the cultured retinae and Müller cells in the deprived eyes

The characteristic apoptotic changes were chromatin condensation, nuclear disintegration and apoptotic body development, with brown-yellowish staining labeled by TUNEL detection. In the cultured retinae, TUNEL-positive nuclei were detected in the ganglion cell layer (92.5%±8.3%) and inner nuclear

layer (46.8%±9.1%), but not in the outer nuclear layer in 100 цM L-DOPA treatment (Figure 2.4). In other groups, no TUNEL-positive nuclei were detected (Figures 2.1, 2.2, 2.3).

For passage 2, retinal Müller cells developed a fibroblast-like morphology with large applanate cell bodies, in the shape of triangles, polygons or fusiform. After 100 μM L-DOPA treatment, many retinal Müller cells were apoptotic, and the apoptotic rate was (59.36±11.25)%(Figure 3.4). In other groups, no TUNEL-positive nuclei were detected in the cultured Müller cells (Figures 3.1, 3.2, 3.3).

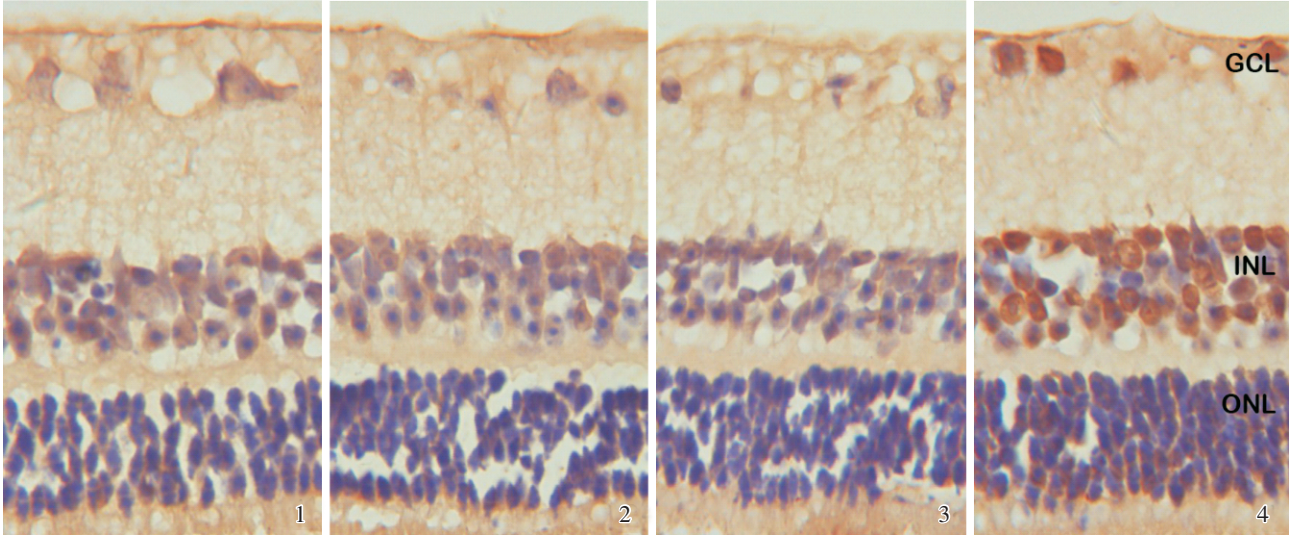

Figure 2 After 100 μM L–DOPA treatment, TUNEL-positive nuclei were detected in the retinal ganglion cell layer (GCL) and inner nuclear layer (INL), but not in the outer nuclear layer (ONL) in the deprived eyes (Figure 2.4). TUNEL×400

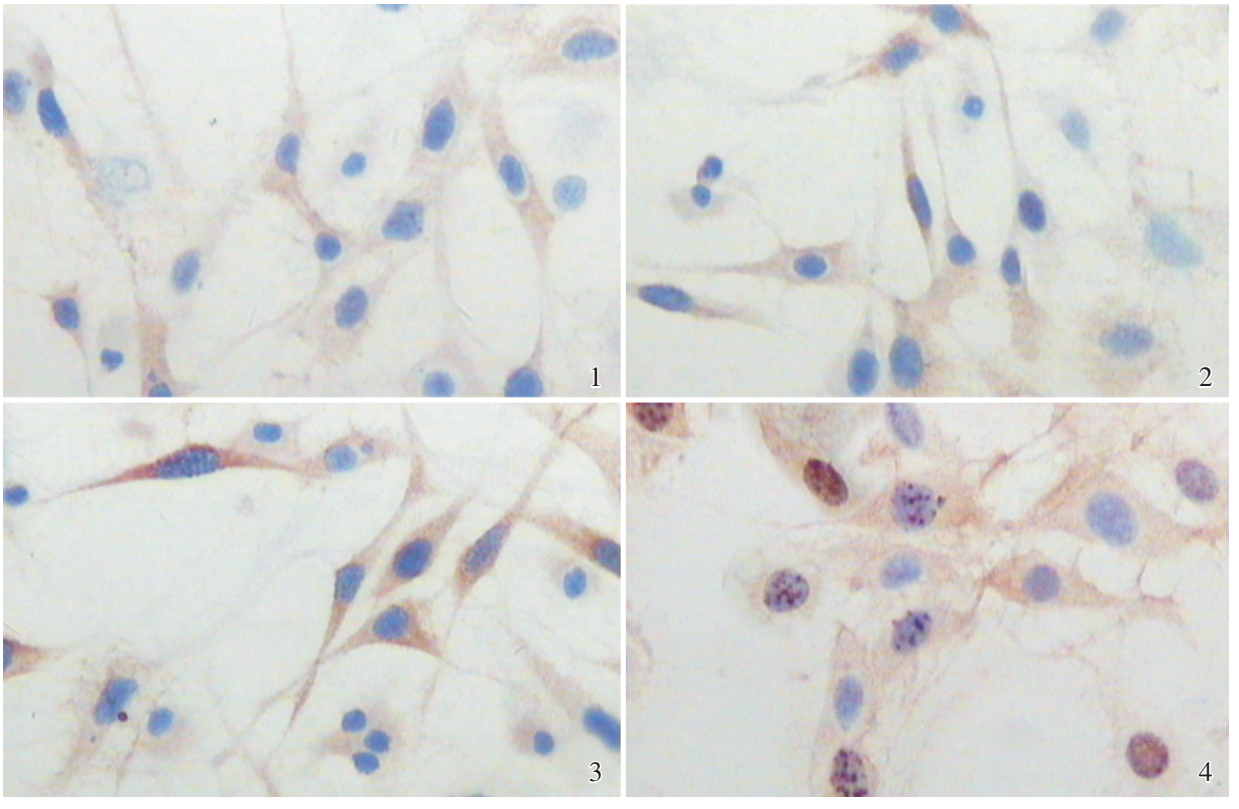

Figure 3 After 100 μM L-DOPA treatment, TUNEL-positive nuclei were detected in the cultured retinal Müller cells in the deprived eyes (Figure 3.4). TUNEL×400

Discussion

In this study, our data showed that exogenous L-DOPA could cause an increase in retinal dopamine in the guinea-pig deprived eyes *in vitro*, and Müller cells were involved in the increase of retinal dopamine. Our findings also suggested an involvement of retinal dopaminergic function in the development of form-deprivation myopia in guinea pigs.

L-DOPA, a precursor of dopamine, can be converted into dopamine in the presence of aromatic L-amino acid decarboxylase(L-AAAD), and dopamine which forms from exogenous L-DOPA has physiological activity and can *in vivo* activate dopamine receptors to implement corresponding physiological functions[17,18]. It has been found that molecular characteristics of dopaminergic neurons are expressed in Müller cells[19], amacrine cells[20], and the inner segments of photoreceptor cells[20]. In our previous study, it was shown that repeated intraperitoneal injection of L-DOPA could inhibit the myopic shift owing to occluding goggles and compensate retinal dopamine in guinea pigs[13]. We found that exogenous L-DOPA (10 μM) could cause an increase in dopamine content of the cultured retinae, which was contributive to explaining the mechanism of retinal dopamine caused by L-DOPA intraperitoneal injection in the guinea-pig deprived eyes. However, 100 μM L-DOPA treatment caused no significant effect on retinal dopamine content, which was associated with its toxic effect on retinae in organotypic culture.

Müller cells are the predominant class of glial cells in the vertebrate retina, and their processes surround the majority of retinal neurons of cone and rod cells, bipolar cells and ganglion cells. Together with retinal neurons, they constitute a "neuron-glia" regulatory network that is involved in the functional activities of the retina. It has been shown that retinal Müller cells can synthesize dopamine[14,15], which participates in regulating the occurrence and development of myopia[6-9]. In the present study, L-DOPA treatment significantly increased the retinal dopamine content in the cultured Müller cells, which showed that Müller cells are a significant source for the increase of retinal dopamine content induced by exogenous L-DOPA.

At present, L-DOPA has been successfully used in treating Parkinson's disease and children with amblyopia, and it has been demonstrated that L-DOPA is a safe, well tolerated drug for the clinical treatment of human disease. However, its side effects and toxicity could not be neglected. In this study, many apoptotic cells were detected in the cultured retinae and Müller cells treated by 100 μM L-DOPA, which evidenced the toxicity of L-DOPA on retinae, and its toxicity increased with the dose. Consequently, the optimal dose and side effects of L-DOPA treatment require further investigations in experimental myopia.

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