·Basic Research·

Comparison of form-deprived myopia and lens-induced myopia in guinea pigs

Hui Xiao[1,2], *Zhong-Yi Fan*[2], *Xiao-Dan Tian*[3], *Yan-Chun Xu*[1]

[1]Department of Ophthalmology, the First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China
[2]Department of Ophthalmology, the 463th Hospital of the People's Liberation Army, Shenyang 110042, Liaoning Province, China
[3]Department of Ophthalmology, the Fourth People's Hospital of Shenyang, Shenyang 110000, Liaoning Province, China
Correspondence to: Yan-Chun Xu. Department of Ophthalmology, the First Affiliated Hospital of China Medical University, Shenyang 110001, Liaoning Province, China. xuyanchun45@sina.cn
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Abstract

• AIM: To study the efficacy difference between form-deprived myopia (FDM) and lens-induced myopia (LIM), the degree of myopia, axial length and pathological changes of the posterior sclera from guinea pigs were evaluated.

• METHODS: Four-week pigmented guinea pigs were randomly assigned into 3 groups, including normal control (n=6), FDM group with monocular cover (n=11) and LIM group with monocular -7D lens treatment (n=11). FDM group was form-deprived while LIM group was lens-induced for 14d. Refractive error and axial length were measured prior to and post treatment, respectively. Morphological changes of sclera were examined using both light and electronic microscopes.

• RESULTS: After 14d treatment, refractive errors for FDM group and LIM group were -3.05±0.71D and -2.12±1.29D, respectively, which were significantly more myopic than that of normal controls and fellow control eyes (P<0.01). As for axial length, it was 7.93±0.03 mm for FDM group and 7.89±0.06 mm for LIM group, which were significantly longer than both normal and fellow controls (P<0.01). With respect to both refractory error and axial length, the differences between FDM group and LIM group were not significant (P>0.05). Under light microscope, both FDM group and LIM group showed thinned sclera, disarrangement of fibrosis and enlarged disassociation between fibers. Consistently, ultrastructural examination showed degenerated fibroblasts and thinned fibers in posterior sclera.

• CONCLUSION: Following two weeks of myopia induction in guinea pigs, with regard to the degree of myopia, axial length and pathological alterations, there was no significant difference between FDM and LIM models. Therefore, FDM and LIM are equally effective and useful as a model of experimental myopia and guinea pigs are ideal animals for induction of experimental myopia because their high sensitivity to both form-deprivation and lens-induction.

• KEYWORDS: form-deprived myopia; lens-induced myopia; pathology
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INTRODUCTION

Worldwide, the incidence of myopia is increasing gradually and 30% of the whole population is suffering from myopia. Certain serious myopia syndromes can cause blindness. The establishment of experimental myopia provides a fundamental model for the investigation of myopia etiology. Experimental myopia is only induced in the laboratory, which is now widely used to study human myopia because anatomical structure and characteristics of refraction of some animals are similar to human spontaneous myopia. A variety of animals, including chick, primate, tree shrew, mouse, cat and guinea pig, have been used to investigate the mechanisms of axial myopia [1-4]. Both form deprivation and lens induction could elongate axial length of eyes and thus caused myopic refraction of various animals[5-7]. With respect to the underlying cellular mechanisms, form-deprived myopia (FDM) and lens-induced myopia (LIM) are two different types of experimental myopia. FDM is induced by forbidding animals to see whereas LIM is induced through wearing concave lens before animals' eyes to disturbing image formation behind the retina, which induces

excessive accommodation and extension of axial length, thereby leading to axial myopia[8,9].

FDM differs from LIM in many ways [10-12]. Previous studies have shown that FDM caused local alterations in retina and usually was not associated with the central nerve system. However, LIM could be inhibited by breaking the circadian rhythm, which was not the case for FDM. As for their association with circadian rhythm, the differences between FDM and LIM were confirmed by the recovery of myopia through holding the circadian rhythm. The identical results in FDM and LIM were refractive error, extending axial length and abnormal growth of the sclera [13]. However, to our best knowledge, there are few reports about the differences between these two types of myopia models under the same experimental conditions, such as experimental duration and environment. The current study compares FDM and LIM of guinea pigs under the same experimental conditions by assessing the degree of refractive error, axial length and morphological alterations of the sclera.

MATERIALS AND METHODS

Materials The animal research protocol used in this study was approved by Experimental Animal Center and Ethnics Committee in China Medical University. All experimental procedures were under Declaration of Helsinki. Twenty-eight pigmented guinea pigs, approximately 4wk old, were obtained from the Experimental Animal Center, China Medical University. All animals underwent biometric measurement for refraction and axial length prior to the experiment. To evaluate the association between myopic development and treatment duration, all animals in the experimental groups underwent biometric measurement after 14d treatment following removal of the facemasks and lenses. As for the normal control group, all animals underwent biometric measurement at the same time point.

Methods Guinea pigs were randomly divided into three groups: FDM (n=11), LIM (n=11) and normal control (n=6). LIM group included LIM refraction eyes (one eye wearing contact lens, 11 eyes) and LIM fellow control eyes (the other eye without contact lens, 11 eyes) while FDM group included FDM refraction eyes (one eye wearing opaque eyeshade, 11 eyes) and FDM fellow control eyes (the other eye without any intervention, 11 eyes). Normal controls were not intervened except for eyes related with examination. The animals were raised for 14d and then measured, and specimens were processed.

FDM was induced by applying monocularly deprived facemask as previously described [6]. LIM was induced by cutting Velcro belt into a ring with 2 cm in diameter, and then creating a hole with 0.8 cm in diameter in the middle of the ring and inserting PMMA contact lens (Lens diameter 13.5 mm, optical diameter 10.5 mm, base curve 9.6 mm, diopter -7D) into the hole with convex plane facing outwards. PMMA contact lens was placed in front of a random eye by gluing Velcro belt on the animal with celloidin (Figure 1). The animals were specially cared by animal care personnel in the room with 14/10 hour light cycle at 20-25℃. The animals were checked once every two hours in the daytime to clean the dirty lens with swab or to refix lens in front of the eye if needed. Lenses and facemasks were demounted 14d after treatment and animals were processed for further experiment.

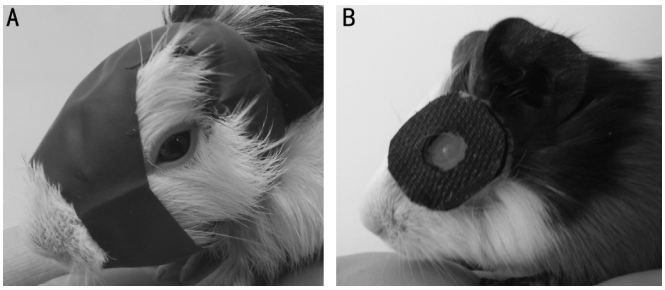

Figure 1 FDM (A) and LIM (B) in guinea pigs.

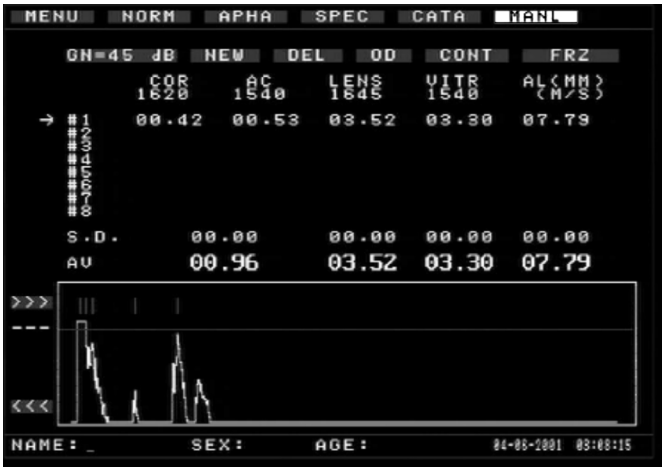

Figure 2 Results of the A-scan of the axial length of guinea pig´s eyes.

Refraction examination and eye axial length measurement were performed before and after myopia induction, guinea pigs were administered with 10 g/L tropicamide- phenylephrine ophthalmic solution on both eyes' conjunctival sac four times with 5min interval, and then eyes were examined with streak retinoscopy in the darkroom. Meanwhile, the axial length of both eyes were also manually measured 10 times by A-scan ultrasonography (Maida Corporation, China) with accuracy at 0.01 mm and then mean values were calculated for analysis (Figure 2). All procedures were performed following anaesthetizing guinea pigs with 0.4% oxybuprocatine (1/5min, 2-3 times).

Specimens processing Eyeballs were extracted under aseptic condition after guinea pigs were sacrificed. Front part

of eye tissue and vitreous body were removed by splitting eyeball along ora serrata under surgery microscope (Topcon, Japan). The sclera tissue was stained by Hematoxylin & Eosin and observed under Olympus BH-2 microphotoscope to examine histopathological changes. Hitachi H-600 electric microscope was used to study the ultrastructural changes of sclera fiber.

Statistical Analysis The refractive status and axial length were compared among the different groups with one-way ANOVA after Bonferroni correction (SPSS Version 16.0, IBM SPSS, USA). Absolute values of biometric results were also compared among the different groups using the same statistical analysis. The difference was defined as significant at $P<0.05$ and highly significant at $P<0.01$.

RESULTS

As for refractive error and eye axial length, there were no statistical significance among LIM, FDM and normal control groups before intervention and both eyes of guinea pigs did not show significant difference ($P>0.05$ for all, Table 1). All eyes were under hypermetropia status prior to the experiment. Fourteen days after treatment, refractive errors for FDM group and LIM group were -3.05±0.71D and -2.12 ±1.29D respectively, which were significantly more myopic than that of normal control and fellow control eyes ($P<0.01$ for all). There was no significant difference between FDM and LIM groups ($P>0.05$ for both, Table 2, Figure 3). With respect to axial length, it was 7.93±0.03 mm for FDM group and 7.89 ±0.06 mm for LIM group, which were significantly longer than that of normal control group and fellow control eyes ($P<0.01$ for all, Table 3, Figure 4). As for both refractive error and axial length, there were no significant differences between FDM and LIM groups ($P>0.05$).

Under light microscope, both FDM and LIM groups showed thinned sclera, disarrangement of fibrosis and enlarged disassociation between fibers (Figure 5). Furthermore, thinner diameter of the posterior sclera collagen fiber and vacuolar degeneration in fibroblasts was observed using electronic microscope (Figure 6).

Table 1 Refraction and axial length of the experimental animals before experiment $\bar{x}$±SD

Groups	*n*	Refraction (Diopter)	Axial length (mm)
Experimental	28	2.71±1.29	7.63±0.04
Fellow	28	2.57±1.31	7.64±0.04

Table 2 Refraction of three groups post-experiment $\bar{x}$±SD

Groups	*n*	Refraction (Diopter)	
		Experimental	Fellow
NOR	6	3.15±0.94	3.25±1.31
FDM	11	-3.05±0.71[b]	2.29±1.01
LIM	11	-2.12±1.29[b]	1.92±1.05

[b]$P<0.01$ *vs* normal control group.

Table 3 Axial Length of three groups post-experiment $\bar{x}$±SD

Groups	*n*	Axial length (mm)	
		Experimental	Fellow
NOR	6	7.75±0.03	7.75±0.04
FDM	11	7.93±0.03[b]	7.75±0.02
LIM	11	7.89±0.06[b]	7.75±0.03

[b]$P<0.01$ *vs* normal control group.

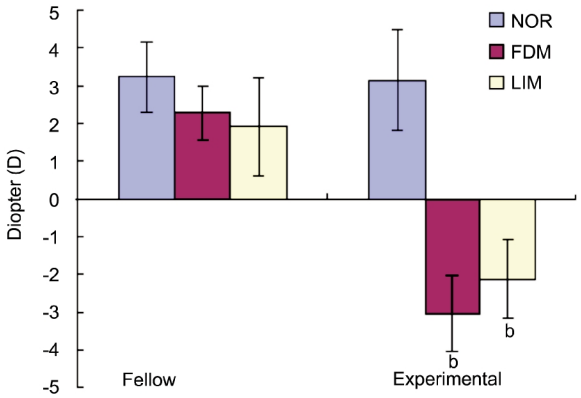

Figure 3 Comparison of refraction post experiment [b]$P<0.01$.

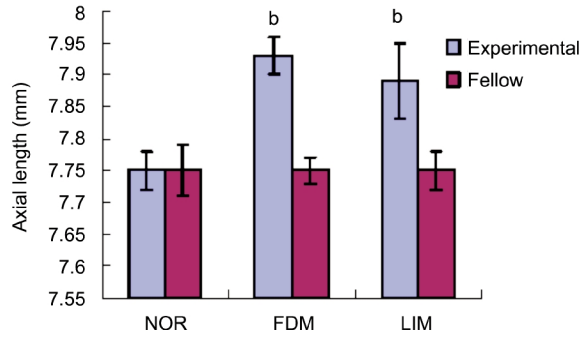

Figure 4 Comparison of axial length post experiment [b]$P<0.01$.

DISCUSSION

To the best of our knowledge, the current study for the first time, under the same experimental conditions, compared the differences of axial myopia between two models of experimental myopia of guinea pigs by investigating the degree of myopia, axial length and pathological changes. Our study showed that both form deprivation and lens induction could elongate eye axial length and cause myopic refraction effectively.

With the development of molecular biology, the studies of myopia etiology have been widely carried out and been well-developed especially after the introduction of the FDM animal model by Wiesel and Raviola in 1977 [7]. Axial elongation caused by myopia could be induced in developing animals by visual form deprivation or by raising the animal with negative lenses in place [6,14-16]. The mechanisms of myopia development were further investigated. However, previous studies showed controversial results about the animal models. The usual applying models were avian (chick) and mammalian (tree shrews or primate). Newly-

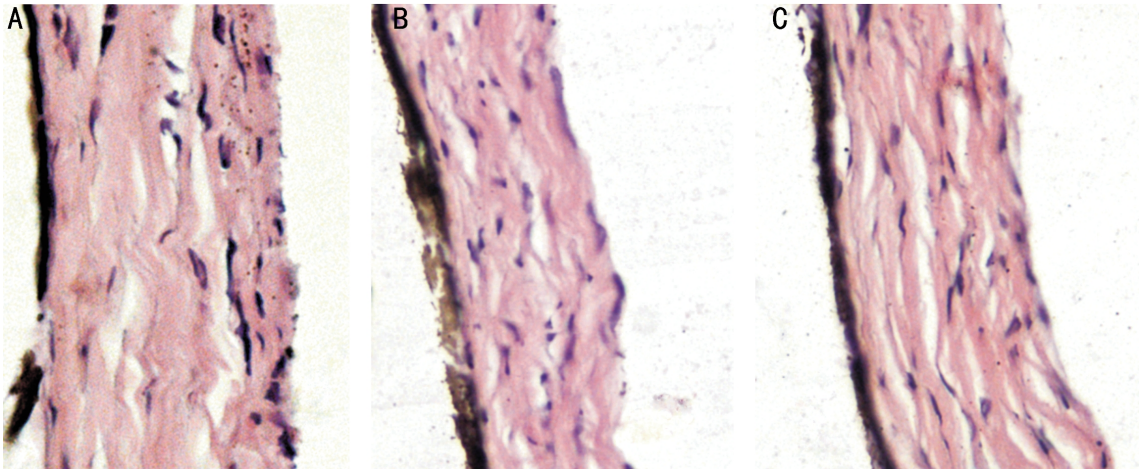

Figure 5 Representative H.E pictures of posterior scleral fibers of normal control (A), FDM (B) and LIM (C), ×200.

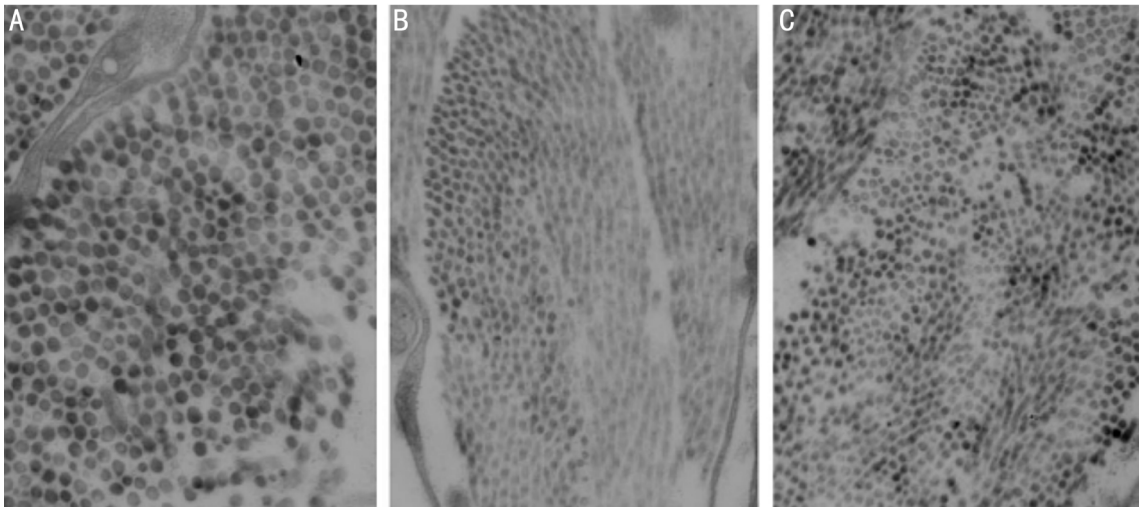

Figure 6 Representative ultrastructural pictures of posterior scleral fibers from normal control (A), FDM (B) and LIM (C), ×6000.

hatched chickens were most susceptible to myopic development as myopia ranging from -4 to -6 diopters could be induced within 1wk of form deprivation [17,18]. The experimental -8.06D myopia was induced by semitransparent goggles for four days and -10.58D myopia was induced by -15D lens for 11d[19,20]. In the chick eyes, the sclera consisted of an inner cartilaginous layer and an outer fibrous layer, and eye elongation involved the increase in total protein accumulation by proteoglycan synthesis and proteoglycan accumulation and growth of the inner cartilaginous layer[21-23]. However, in the mammalian eyes, in which the sclera comprises a single fibrous layer, ocular expansion did not involve net growth rather than a remodeling of the sclera with decreased proteoglycan synthesis and a net loss of scleral tissue [24]. In addition, the accommodating muscle of chick was not smooth muscle but striated muscle and the receptor not nicotine but hydroxycholine. The mechanics of accommodation and the innervation were different compared to mammalian eyes. Therefore, chick is not an ideal model for the study of human myopia due to the differences between human and chicken regarding the visual axis, ocular anatomy and accommodation mechanisms. Primates are the most ideal models for human myopia as they are most similar to human in terms of ocular anatomy and physiology [25,26]. However, availability of primates is very limited and the cost is higher than other animals. Moreover, the myopia induced in primates generally requires a longer duration than other animals. As an alternative to the primate model, tree shrews and guinea pigs could develop myopia ranging from -3 to -6 diopters within 1wk of form deprivation [27]. As for mice, they were hyperopic and their eyes were shorter at the end of the lid-suture period, therefore they developed myopia only when the lid-suture was discontinued and form vision was restored. Furthermore, mice were unlikely to have accommodation since they lacked ciliary muscles in their eyes [28]. Previous study also showed that form deprivation in cats did not consistently induce axial myopia[29]. Therefore, guinea pigs appear to be a suitable animal model for the study of human myopia.

Guinea pigs were applied widely as experimental animals in biomedical studies. They are an ideal model because of low cost, mild temper and easy raise. Moreover, the ocular

anatomic structure, biologic structure and physiological function of guinea pigs were similar to human and primates. Because guinea pigs were sensitive to form-deprivation and lens-induction, they were applied in myopia study recently [30,31]. High myopia could be induced in short time and had emmetropia process postnatally. McFadden *et al*[3] reported form deprivation induced -6.6D myopia in five-day old guinea pigs and prolonged axial length by 0.146 mm. Form deprivation also induced -5.8D myopia and prolonged axial length by 0.170 mm in two-day old guinea pig. Lu *et al*'s [6] study showed that form-deprived 3-wk guinea pig for 14d induced -2.2D myopia. Consistently, our study showed that 14d of form deprivation on four-week old guinea pigs induced -3.05D myopia and prolonged 0.18 mm of axial length. Furthermore, a -7D concave lens placed over the eye induced -2.12D and prolonged 0.14 mm.

After 14d treatment, refractive errors for FDM group and LIM group were -3.05±0.71D and -2.12±1.29D, respectively. Significant differences, compared with that of normal control group and fellow control eyes, were observed. In terms of axial length, it was 7.93±0.03 mm for FDM group and 7.89± 0.06 mm for LIM group. With respect to both refractive error and axial length, significant differences between FDM group and LIM group were not presented. However, there were no significant differences among three fellow groups which showed axial myopia induced by FD and LI. There were no difference in the diopter and axial length following fourteen days treatment.

Under light microscope, both FDM group and LIM group showed thinned sclera, disarrangement of fibrosis and enlarged disassociation between fibers. Strikingly, posterior sclera demonstrated degenerated fibroblasts and decreased fibers. The sclera is the outer coating of the eyes which, in addition to protecting the retina and allowing the attachment of the extraocular muscles, controls the size of the eyes and the location of the retina relative to the focal plane. Sclera is made up with fibroblasts and the collagen bundle which parallel the eye wall under electric microscope. The diameter of the posterior sclera fibroblasts was thinner and the disarranged fibrosis was enlarged compared with that from normal control group and there was vacuolar degeneration in the fibroblasts. Previous studies showed that animals' eyeballs had subjective emmetropia process after birth [17,31]. Moreover, Liu *et al* [32] reported that the chief differences at posterior sclera were diameter and morphology of fibroblasts between normal and myopia eyes, which were parallel with the results of our study. In terms of the degree of myopia after two-week induction, there was no significant difference between FDM and LIM. Although the underlying mechanism of myopia formation was not identical, the induced myopia was similar.

FDM was induced by wearing facemask and LIM was induced by placing Velcro belt. The Velcro belt was glued on the animal fossa orbitalis with celloidin. Both methods had the advantages which don't oppress the eyelid and eyeball, and removal and operation were easy without need of anesthesia. Our results were consistent with previous studies regarding the myopia diopter and induced axial length [6,30]. Moreover, the eyes from guinea pigs and human being share similar anatomical structure because both of them are mammal. The methods used here are suitable and effective to construct myopia models. Guinea pigs are an ideal model for induction of experimental myopia because they are sensitive to form-deprivation and lens-induction, both of which induce axial myopia. In our study, the models of experimental myopia established are operation-friendly without the necessity of anaesthesia.

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