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Effects of glucocorticoid on the eye development in guinea pigs

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ABSTRACT

Glucocorticoid (GC) has been widely used in clinic. However, the effect of GC on normal and myopic development of eyes is still unknown. In this study, 3-week-old guinea pigs were randomly divided into four groups: No-Lens (control), GC + No-Lens, negative lens-induced myopia (LIM), and GC + LIM. To induce myopia, right eyes were covered with a −10 D lens in GC + LIM and LIM groups. GC + No-Lens and GC + LIM groups received intraperitoneal injections of hydrocortisone (10 mg/kg) once daily for 2 weeks, and then received intragastric hydrocortisone (32.5 mg/kg) every other day for the next 4 weeks, while No-Lens (control) and LIM groups were injected intraperitoneally with saline for 2 weeks, and then given saline by intragastric administertion for the next 4 weeks. Several parameters were assessed: ocular axial length and refractive error, sclera thickness, matrix metalloprotein-2 (MMP-2) and tissue inhibitor metalloprotease-2 (TIMP-2) expressions and localization of the posterior sclera, plasma concentrations of free triiodothyronine (FT3), free thyroxine (FT4), testosterone (T), and oestradiol (E2). Results indicated that: (1) in normal eye development, hydrocortisone could inhibit both the axial elongation and the myopic shift; whereas (2) in LIM eye development, hydrocortisone (a) enhanced the axial elongation, myopic shift and sclera thinning; (b) enhanced the MMP-2 expression and decreased TIMP-2 expression, and (c) elevated the plasma concentration of E2 but decreased the levels of FT3, FT4, and T. In conclusion, glucocorticoid may influence both normal and LIM eye development. The balance of the hormones is fundamental for the eye development.

1. Introduction

Glucocorticoids (GCs) are steroid hormones that regulate homeostatic functions, exhibit immunosuppressive, anti-inflammatory and anti-angiogenic properties, and are indispensable for life. Physiological GC levels are maintained by a negative-feedback loop that controls the hypothalamic–pituitaryadrenal axis [1,2]. GCs are frequently and extensively used in routine clinical practice, and they have a variety of effects on many conditions, such as rheumatoid arthritis, asthma, allergic purpura, and systemic lupus erythematosus [1,3]. These conditions can be grouped into those that require either high-dose or low-dose treatment. For example, in emergency situations, such as acute kidney transplant rejection, a high dose of GC is generally administered by intravenous injection. By contrast, for chronic disease, such as nephrotic syndrome, high-dose GC treatment is administered initially, and then low-dose maintenance therapy with GC is given orally [3].

At present, GC is also extensively used to treat other childhood diseases besides asthma, such as childhood steroid-sensitive nephrotic syndrome (SSNS), a kidney disease that responds well to high-dose GC therapy, but with frequent relapses, so that it requires prolonged therapy to induce remission [4]. Long-term therapy with GC is nearly always accompanied by undesirable side effects, e.g., osteoporosis, hypertension, diabetes mellitus, atrophy of skin and muscle, glaucoma, cataracts, and increased susceptibility to infections [5,6].

The childhood period is vital for eye development. At this stage, eye development is easily affected by various factors. For instance, it has been shown that myopia progression is sensitive to many predisposing risk factors, including genetic factors and environmental factors [7] such as near work [8], education level [9] and time spent outdoors [10]. It is noted that experimental manipulations of visual experience influence the eye growth and refractive status in many species including chicks, tree shrews, mice, monkeys, and guinea pigs [11]. At present,

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the guinea pig (Cavia porcellus) has been frequently used for modeling human diseases and has been increasingly used for myopia research. The biometric changes in developing guinea pig eyes are more like those of monkeys than to the widely used chicken models of myopia under similar experimental conditions. In guinea pigs, hyperopia is present at birth and rapidly reduces to emmetropia within the first 3 weeks (21 days) of age [12]. Regarding the development of myopia, there is a large number of evidences showing that myopia development is correlated with excessive optic axial elongation, which is accompanied by the reduction of scleral stiffness and biomechanical strength. These scleral alterations include decrease in collagen expression, increases in collagen turnover, myofibroblast transdifferentiation and matrix metalloproteinase (MMP) up-regulation as well as extracellular matrix remodeling [13].

In clinical practice, GC has been widely used to treat childhood diseases; however, it is still unclear whether GC affects eye development. Therefore, in the present study, we established a negative lens-induced myopia (LIM) guinea pig model to investigate the effect of GC on the development of LIM. The present study aimed to address this issue using guinea pigs with either normal eye development or LIM in the presence and absence of GC treatment.

2. Materials and methods

2.1. Animals

Three-week-old pigmented male guinea pigs (Cavia porcellus) were provided by Henan Kangda Laboratory Animal Co., Ltd. and housed in plastic boxes with free access to food and water at a constant temperature of 22 °C [14–16], and on a 12 h light: 12 h dark schedule. The average light intensity on the floor of the cage was 350 lx [17]. The project was approved by the Animal Care and Use Committee of Shandong University of Traditional Chinese Medicine (2013-003), and all procedures abided by the ARVO Statement for the Use of Animals in Ophthalmology and Vision Research.

2.2. GC administertion and LIM fabrication

Our experiment included two parts. Part 1: to explore the effects of GC on the axial length and refraction of normal and LIM guinea pigs after treatment for 0, 2, 4, or 6 weeks (n = 8 per group); Part 2: to investigate the effects of GC on LIM guinea pigs at the 2-week interval (n = 32 per group). As shown in Fig. 1, the guinea pigs were randomly assigned to the following four groups: No-Lens (control), GC + No-Lens, lens-induced myopia (LIM) and GC + LIM. To induce myopia, right eyes of the GC + LIM group were covered with a −10 D lens, and neither eye of the GC + No-Lens group received any intervention. GC + No-Lens and GC + LIM groups received intraperitoneal injections of hydrocortisone (Tianjin Jiaozuo Pharmaceutical Company, Tianjin, China) at a dose of 10 mg/kg once daily (8:00–10:00 a.m.) for 2 weeks and then received intragastric administertion of hydrocortisone at a dose of 32.5 mg/kg every other day for the next 4 weeks to maintain the treatment effect [18]; Guinea pigs in No-Lens and LIM groups were injected intraperitoneally with the same volume of saline at the same time for 2 weeks and were then given saline by intragastric administertion for the next 4 weeks. During this period, the right eyes of the LIM group were covered by a −10 D lens, whereas neither eye of the No-Lens (control) group received any intervention. Lenses were mounted onto self-made frames using surgical tapes and glued onto the right eyes of guinea pigs [19] and cleaned each morning and evening to prevent form-deprivation effects. The axial lengths and refraction in all groups were measured at the four intervals (0, 2, 4, 6 weeks).

2.3. Refractive and A-scan measurements

In this study, streak retinoscopy and A-scan ultrasonography were used to measure the refraction and axial length, respectively. First, one drop of 1% cyclopentolate hydrochloride (Alcon, USA) was topically instilled into the conjunctival sac of guinea pigs every 5 min three times to attain a completely dilated pupil and cycloplegia. The retinoscopy for all animals was performed consistently at a distance of 20 cm by the same optometrist with a hand-held streak retinoscope (YZ24, 66 Vision Tech. Co., Ltd, China) in a dark room. The refraction of the eyes was defined as the mean value of the refractive errors along the vertical and horizontal meridians [15,20] of three repeated measurements.

The axial length was defined as the distance from the corneal surface to the retinal pigment epithelium [21] and was measured by A-scan ultrasonography (Cinescan, Quantel Medical, France). The frequency of the ultrasonic probe emission was 11 MHz41. The conducting velocities were 1557.5 m/s in the anterior chamber, 1723.3 m/s in the lens and 1540 m/s in the vitreous chamber [22–24]. A topical anaesthesia of oxybuprocaine hydrochloride (Santen Pharmaceutical, Japan) was administered before the axial length measurements. The tip of the probe slightly touched the optical center of the cornea during the measurements. The results were obtained from the mean value of 10 repeated measurements, which minimized the effect of an obvious outlier value.

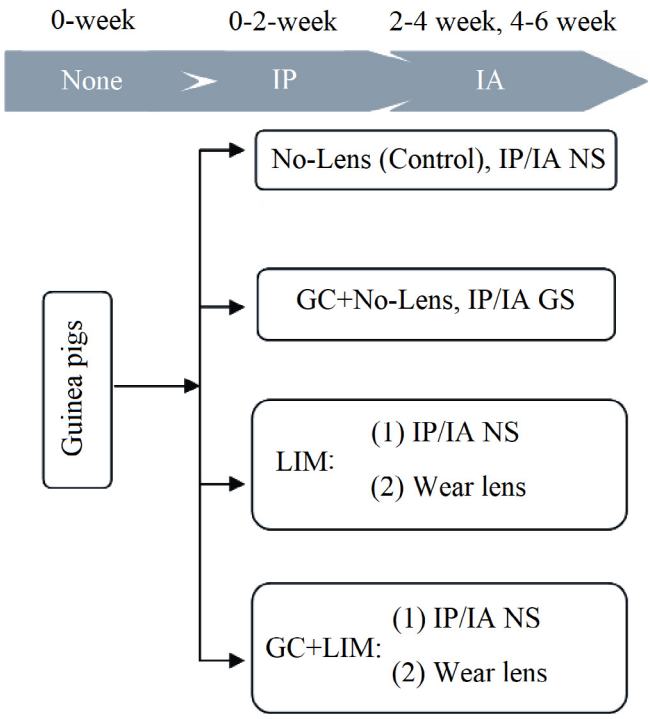

Fig. 1. Illustration of GC administration and lens-induced myopia. IP: intraperitoneal injection. IA: intragastric administration. NS: normal saline; GC: glucocorticoid; GC + No-Lens, glucocorticoid + No-Lens; LIM, lens-induced myopia; GC + LIM, glucocorticoid + lens-induced myopia.

2.4. Haematoxylin and eosin (H&E) staining

The guinea pigs were euthanized by injection of a lethal dose of additional anaesthetic (phenobarbital), and the eyes were extracted and fixed without opening them by immersion in 4% freshly prepared paraformaldehyde in 0.1 M phosphate-buffered saline (PBS; pH = 7.4) for 24 h at 4 °C. Subsequently, the eyes were opened from the corneoscleral limbus (Fig. 2A), embedded in paraffin and marked at the 12o'clock position on the corneal limbus. The tissue was sectioned at 4 μm from the marks along the anterior–posterior axis, and then the sections were stained with haematoxylin and eosin (H&E) and observed under an optical microscope (Eclipse55i, Nikon, Japan).