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Scleral cross-linking using riboflavin and ultraviolet-A radiation for prevention of progressive myopia in a rabbit model

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ABSTRACT

Our study demonstrates the effect of scleral cross-linking using riboflavin and ultraviolet-A radiation on the development of axial myopia in a rabbit model. Axial length of the eyeball was measured by A-scan ultrasound in 22 New Zealand white rabbits aged 13 days. The right eyes then underwent 360-degree conjunctival peritomy with (experimental group, $n = 11$) or without (control group, $n = 11$) scleral cross-linking, followed by tarsorrhaphy. The left eyes served as a control eye. In the experimental group, the right eyeballs were divided into quadrants, and every quadrant had either 2 ($n = 8$) or 6 ($n = 3$) scleral irradiation zones, each with an area of 0.2 cm^2 and radius of 4 mm. Cross-linking was performed by dropping 0.1% dextran-free riboflavin-5-phosphate onto the irradiation zones at 20 s before ultraviolet-A irradiation and every 20 s during the 200-s irradiation time. UVA radiation (370 nm) was applied perpendicular to the sclera at 57 mW/cm^2 (total UVA light dose, 57 J/cm^2). Tarsorrhaphies were removed on day 55, followed by repeated axial-length measurement.

In the control group, mean axial length in the right eyes increased from 10.50 ± 0.67 mm at baseline to 15.69 ± 0.39 mm 55 days later, for a mean change of 5.19 ± 0.85 mm. In the experimental group, corresponding values were 10.68 ± 0.74 mm and 14.29 ± 0.3 mm, for a mean change of 3.61 ± 0.76 mm. The between-group difference in the change in mean axial length was statistically significant ($p < 0.001$, Mann–Whitney nonparametric test).

The present manuscript demonstrates that scleral cross-linking with riboflavin and ultraviolet-A radiation effectively prevents occlusion-induced axial elongation in a rabbit model.

1. Introduction

The reported prevalence of myopia in the general population is about 30% in the USA and up to 60% in Asian countries, and appears to be increasing worldwide (McBrien and Gentle, 2003; Saw et al., 2002). Individuals with myopia, especially high myopia, are at higher-than-normal risk of cataract, glaucoma, retinal detachment, chorioretinal abnormalities, and optic disc anomalies. In addition, pathological myopia is a common irreversible cause of visual impairment and blindness (Rose et al., 2001; Saw et al., 2005; Tano, 2002).

The etiology of myopia is still controversial, and its treatment continues to pose a challenge. Genetic factors have been widely considered, but phenotype penetration is unpredictable (Gilmartin, 2004; Morgan and Rose, 2005). Intense and prolonged near work has been found to cause sensory-input-related scleral changes (Baldwin, 1981; McBrien and Gentle, 2001), and visual deprivation due to cataract or ptosis can lead to myopia via a disruption in the feedback mechanism of emmetropization (Weiss, 2003). In animal studies of myopia, unilateral sight reduction was induced by eyelid suturing (Greene and Guyton, 1986; McBrien and Norton, 1992; McKanna and Casagrande, 1978; Shapiro, 1981), placement of an occluder at a short distance from the eye, corneal tattooing (Wiesel and Raviola, 1979). Attachment of semiopaque or transparent contact lenses onto the scleral-corneal cup, developed eye

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elongation with high myopia in the obstructed eyes (Smith et al., 1980). In contrast, the fellow eyes, unimpaired by vision manipulation, grew normally. However, for artificial myopia to occur in these studies, the occlusion process has to be performed on very young animals, as no sight deprivation experiments carried out on adult specimens have proved successful.

Therapeutic attempts to arrest myopic progression include the administration of cycloplegic or hypotensive drugs such as atropine, tropicamide, pirenzepine, or timolol, use of optical correction to assist in near work and accommodation (Saw et al., 2002; Shapiro, 1981; Wildsoet and Norton, 1999), and microsurgical scleroplasty (Avetisov et al., 1997; Thompson, 1978; Whitmore and Curtin, 1987). The success of these methods is controversial. No efficient means of preventing progressive myopia has been found to date.

Myopia leads to progressive thinning of the sclera owing to a decrease in collagen fiber diameter (Curtin et al., 1979; Liu et al., 1986) and disturbances of collagen fibrillogenesis (Funata and Tokoro, 1990). These changes result in an increase in the biomechanical extensibility and "creep" of the sclera (McBrien and Gentle, 2003; Rada et al., 2006). Studies have shown that impaired collagen cross-linking is an important factor in the weakening process of the myopic sclera (McBrien and Norton, 1994; Mechanic, 1972; Iomdina et al., 1993). Wollensak et al. (Wollensak et al., 2003; Wollensak and Spörl, 2004; Wollensak et al., 2005) induced collagen cross-linking by applying the photosensitizer riboflavin and ultraviolet-A (UVA) irradiation (370 nm) and noted a significant, 157% increase in the rigidity of porcine and human sclera in vitro (Wollensak et al., 2003) and a 465% increase in rabbit scleral rigidity in vivo (Young's modulus) (Wollensak and Spörl, 2004). Cross-linking also had a long-term effect on rabbit sclera in vivo: rigidity increased by 320.4% after 3 days, 277.6% after 4 months, and 502% after 8 months (Young's modulus) (Wollensak and Iomdina, 2009).

On the basis of these findings, we hypothesized that scleral cross-linking may serve as a means for sclera-based treatment of myopic progression. The aim of this study was to determine if scleral collagen cross-linking prevents the development of axial myopia induced by visual axis occlusion.

2. Materials and methods

2.1. Setting and animals

Twenty-two 13-day-old New Zealand white rabbits weighing 208–350 g were used in the experiment. Animals were treated in accordance with the ARVO Resolution on the Use of Animals in Research. The study protocol was approved by the institutional Committee for Laboratory Animal Research (approval no. 022-4598-2; 021211).

2.2. Procedure

Animals were placed under general anesthesia with an intramuscular injection of 0.1 ml ketamine hydrochloride 100 mg/ml (50 mg/kg) and 0.05 ml xylazine hydrochloride 25 mg/ml (8 mg/kg). Three axial length measurements were performed in each eye after topical anesthesia using an ultrasound A-scanner (Allergan-Humphrey, San Leandro, CA. Instrument accuracy of ±0.034 mm) and then the measurements were averaged. The probe was applied perpendicularly to the central cornea. Half the rabbits underwent 360-degree conjunctival peritomy and tarsorrhaphy in the right eye (control group), and half underwent 360-degree conjunctival peritomy, followed by cross-linking and tarsorrhaphy in the right eye (experimental group). The left eye in both groups was not operated. Fifty-five days after surgery, the tarsorrhaphies were removed, and the axial length measurements were repeated in all eyes.

The animals were sacrificed by intravenous injection of 22% phenobarbital (2 ml), and both eyes were enucleated.

Treated and untreated eyes were dissected and fixed in 4% neutral-buffered formalin, 4 μm-thin paraffin sections were stained with hematoxylin and eosin. The specimens were evaluated using a Olympus light microscope (Olympus BX52TF, Olympus optical Co. Ltd, Japan) at 4–100-fold magnification.

2.3. Cross-linking technique

The right eyeballs in the experimental group were divided into quadrants between the rectus muscles. In 8 rabbits (8 eyes), each quadrant had two irradiation zones, one at the equatorial sclera and one at the posterior sclera (Figs. 1 and 2). In the other 3 rabbits (3 eyes), each quadrant had 6 irradiation zones, as follows: 2 in the posterior sclera, 2 at the equator, and 2 anterior to the equator (Figs. 3 and 4). The area of each zone measured 0.2 cm^2 with a radius of 4 mm. Photosensitizer solution containing 0.1% dextran-free riboflavin-5-phosphate (Concept for Pharmacy Ltd., Kfar Saba, Israel) was dropped onto each irradiation zone for 20 s before irradiation and then every 20 s during the 200-s irradiation period.

Rabbits with two irradiation zones per quadrant had a total irradiation time of 1600 s per eye and the six irradiation zones per quadrant rabbits had a total irradiation time of 4800 s per eye.

The irradiation and the dropping of riboflavin were done simultaneously by two surgeons.

The irradiation device includes a UV A (370 nm) light source connected to a beveled down custom made fiber optic. Following measurement of energy power and calibration the device was set to 57 mW/cm^2.

UVA light (370 nm) was applied perpendicular to the sclera, 57 mW/cm^2.

This method provided a total UVA light dose of 57 J/cm^2. (57 mW/cm^2 on 0.2 cm^2 is 11.4 mW/0.2 cm^2. Using 11.4 mW/0.2 cm^2 for 200 s is a cumulative load of 2.2 J per 0.2 cm^2 spot)

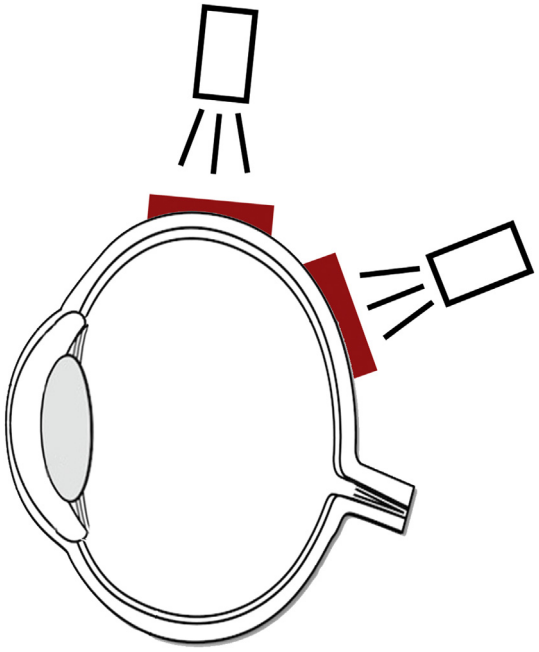

Fig. 1. Coronal plane of the spot distribution in a two irradiation zones per quadrant rabbit. (corresponding dimensions)