Effects of genipin corneal crosslinking in rabbit corneas

Marcel Y. Avila, MD, PhD, Mauricio Narvaez, MD, Juan P. Castañeda, MD

PURPOSE: To evaluate the effect of genipin, a natural crosslinking agent, in rabbit eyes.

SETTING: Department of Ophthalmology, Universidad Nacional de Colombia Centro de Tecnologia Oftalmica, Bogotá, Colombia.

DESIGN: Experimental study.

METHODS: Ex vivo rabbit eyes (16; 8 rabbits) were treated with genipin 1.00%, 0.50%, and 0.25% for 5 minutes with a vacuum device to increase corneal permeability. Penetration was evaluated using Scheimpflug pachymetry (Pentacam). In the in vivo model (20 rabbits; 1 eye treated, 1 eye with vehicle), corneas were crosslinked with genipin as described. Corneal curvature, corneal pachymetry, and intraocular pressure (IOP) assessments as well as slitlamp examinations were performed 0, 7, 30, and 60 days after treatment.

RESULTS: In the ex vivo model, Scheimpflug pachymetry showed deep penetration in the rabbit corneas with an increase in corneal density and a dose-dependent relationship. Corneal flattening was observed in treated eyes (mean 4.4 diopters $\pm$ 0.5 [SD]) compared with the control eyes. Pachymetry and IOP were stable in all evaluations. No eye showed toxicity in the anterior chamber or in the lens.

CONCLUSIONS: Corneal crosslinking induced by genipin produced significant flattening of the cornea with no toxicity in rabbit eyes. This crosslinking could be useful in the treatment of corneal ectasia and in the modification of corneal curvature.

Financial Disclosure: None of the authors has a financial or proprietary interest in any material or method mentioned.

J Cataract Refract Surg 2016; 42:1073–1077 © 2016 ASCRS and ESCRS

Exogenous crosslinking has emerged as a new way to reduce corneal deformation and as a treatment for corneal ectasia and keratoconus.[1] In the clinical setting, crosslinking involves the use of ultraviolet (UV) light and riboflavin; however, UV light induces changes in corneal cells that must be considered when treatment is proposed. Therefore, a methodology that avoids UV light is desirable.[2,3]

Genipin has emerged as a new crosslinking agent with excellent biocompatibility,[4] low toxicity,[5] and the ability to crosslink the cornea[5] and sclera,[6] as shown by in vitro and in vivo myopia model experiments.[7] We also found lower toxicity to endothelial and stromal cells and improved stiffness of the cornea in comparison with UV and riboflavin crosslinking.[6]

We previously found an increase in corneal stiffness compared with accelerated UV crosslinking, similar to the Dresden UV protocol.[8] To make this feasible in the clinical setting, a reduction in the delivery time of the crosslinker genipin would be desirable. Despite the low in vitro and in vivo toxicity of genipin–chitosan membranes in the anterior chamber in animal

Submitted: November 23, 2015.
Final revision submitted: April 5, 2016.
Accepted: April 7, 2016.

From the Department of Ophthalmology (Avila, Narvaez), School of Medicine, Universidad Nacional de Colombia, and Centro de Tecnologia Oftalmica (Avila, Castañeda), Sociedad de Cirugia Ocular, Bogotá, Colombia.

Corresponding author: Marcel Y. Avila, MD, PhD, Department of Ophthalmology, Facultad de Medicina, Universidad Nacional de Colombia, Carrera 30 Calle 45, Bogotá DC, Colombia. E-mail: myavilac@unal.edu.co.

models,[9] further studies are required to provide evidence of anterior segment effects and the effect of corneal flattening in animal models before clinical studies are considered.

In this study, the effects of genipin on the cornea were evaluated using an optimized genipin delivery system. The anterior segment and intraocular pressure (IOP) were measured to elucidate the hypothesis that genipin crosslinking induces a reduction in corneal curvature.

MATERIALS AND METHODS

Ex Vivo Experiments

Eight rabbits (aged 5 months) were obtained from a local abattoir and their eyes were used to assess penetration and cornea densitometry in vitro. Briefly, the fresh rabbit eyes were enucleated and treated with genipin at different concentrations (1.00%, 0.50%, and 0.25%, and vehicle; 4 eyes per group). Genipin was dissolved in dimethyl sulfoxide and then in phosphate-buffered saline pH 8.5 (Challenge Bioproducts Co., Ltd.) with a purpose-built vacuum device (the same used in the in vivo experiments) to increase the permeability of genipin and to avoid contact with other structures. A subset of experiments was performed in which the rabbit eyes were cannulated to obtain an IOP of 18 mm Hg. Then, suction was applied using the vacuum device and the IOP was measured with an applanation tonometer (Tono-Pen, Reichert). Treatment was performed for 5 minutes, after which the eyes were washed with saline and incubated at 30°C for 24 hours. The eyes were then evaluated using the Pentacam Scheimpflug pachymetry module (Oculus Optikgeräte GmbH) from the Scheimpflug images and corneal densitometry.

In Vivo Experiments

Twenty New Zealand albino rabbits (aged 5 months) were used. The rabbits were handled according to the Association for Research in Vision and Ophthalmology protocols for animal experimentation in ophthalmology. Protocols involved paired-eye design (1 eye treated with genipin and the contralateral eye with vehicle) to reduce the number of animals required and interanimal variability.

Under general anesthesia of ketamine (50 mg/kg), xylazine (10 mg/kg), and acepromazine (1 mg/kg), both eyes of each rabbit were deepithelialized and 1 eye was treated with genipin 0.25% in a custom vehicle for 5 minutes using a vacuum device designed to ensure contact between the cornea and the genipin and to prevent drops in the conjunctiva. Briefly, a plastic syringe (5 cm long) was connected to a 0.8 mm plastic cylinder, and a vacuum with 4 cm of negative pressure was applied for 5 minutes. This system was adapted from the principle of the vacuum device described by Myung et al.[A]; the contralateral eye was treated with the vehicle alone.

The cornea was then washed with saline solution, and a mixture of tobramycin and dexamethasone was applied to both eyes every 6 hours for 5 days until complete epithelialization occurred.

Measurements

The Scheimpflug system was used to measure corneal densitometry values. The Scheimpflug system quantifies the density of the cornea on a scale from 0 to 100 (arbitrary optical units). Peak densitometry values were recorded directly from the axis line appearing in the Scheimpflug image in the ex vivo eyes, and the depth was calculated as the percentage of denser areas in the center of the cornea.

Corneal thickness was measured using an ultrasound pachymeter (Pachette 3, DGH Technology, Inc.). Corneal curvature was measured using an autorefractor keratometer (KP 8000, Topcon Europe Medical B.V.). The IOP was measured using an applanation tonometer. Slitlamp evaluations were performed on 0, 1, 5, 15, 30, and 60 days, and changes in the cornea, lens, and anterior segment were evaluated. Changes in corneal transparency (haze) were graded from 0 (no haze) to 5 (severe haze that precluded visualization of the anterior chamber). These measurements were performed in both eyes on 0, 1, 5, 15, 30, and 60 days.

Statistical Analysis

In most instances, the statistical significance of comparisons was established with a paired Student *t* test. Corneal pachymetry, corneal keratometry (K), and IOP were compared using 1-way analysis of variance on ranks; then, statistically significant comparisons were established with the Kruskal-Wallis test using Sigmastat (Systat Software, Inc.). Differences were considered significant if the probability of null hypothesis (*P*) was less than 0.05.

RESULTS

Ex Vivo Corneal Densitometry and Intraocular Pressure Changes

The mean Scheimpflug corneal densitometry was concentration dependent, with a maximum value with genipin 1.00% (mean 95 optical units ± 4.7 [SD]) followed by genipin 0.50% (mean 74 ± 12.3 optical units) and a minimum value with genipin 0.25% (mean 68.5 ± 9.8 optical units) in comparison with the control corneas. In the deep layers of the cornea, this effect was also concentration dependent, affecting 81% of the corneal layers with genipin 1.00%, 38.1% with genipin 0.50%, and 32.2% with genipin 0.25% (Figure 1). The IOP increased from a mean of 18.0 mm Hg to 33.0 ± 2.3 mm Hg with the vacuum device (n = 10).

In Vivo Study

Keratometry The mean K values (20 eyes each group; all timepoints) were and 49.2 ± 0.8 D (genipin) and 49.7 ± 0.6 diopters (D) (control) at baseline, 47.1 ± 0.8 D (genipin) and 47.9 ± 0.3 D (control) at 30 days, and 44.8 ± 0.4 D (genipin) and 46.28 ± 0.5 D (control) at 60 days. The difference between the 2 groups was statistically significant at 60 days ($t(7) = 2.655$, $P = .001$; paired-samples *t* test). The mean reduction in the steepest values was 4.4 ± 0.5 D in the genipin group and 2.5 ± 2.0 D in the control group; the difference was statistically significant ($P = .005$) (Figure 2).

Pachymetry The mean preoperative pachymetry was 412 μm in the genipin-treated eyes and 417 μm in the