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Efficacy, Drug Sensitivity, and Safety of a Chronic Ocular Hypertension Rat Model Established Using a Single Intracameral Injection of Hydrogel into the Anterior Chamber

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Background: Chronic ocular hypertension (COH) models mostly focus on changes in intraocular pressure (IOP) and loss of retinal ganglion cells (RGCs). The present study evaluated important glaucoma-related changes in visual function, response to common ocular hypotensive drugs, and safety for our previously developed rat model.


Material/Methods: The model was established through a single injection of hydrogel into the anterior chambers. Efficacy was assessed through F-VEP by measuring latency and amplitude of P1. We evenly divided 112 rats into 4 groups: control and COH at 2, 4, and 8 weeks. Response to 5 common drugs (brimonidine, timolol, benzamide, pilocarpine, and bimatoprost) were each tested on 6 rats and assessed using difference in IOP. Safety assessment was conducted through histological analysis of 24 rats evenly divided into 4 groups of control and COH at 2, 4, and 8 weeks. Corneal endothelial cells (CECs) of 24 additional rats were used to determine toxic effects through TUNEL and CCK-8 assays.

Results: P1 latency and amplitude of VEP demonstrated the model is effective in inducing optic nerve function impairment. Only the drug pilocarpine failed to have an obvious hypotensive effect, while the other 4 were effective. CECs at 2, 4, and 8 weeks showed no significant differences from control groups in results of histological analysis, TUNEL, and CCK-8 assays.

Conclusions: A single injection of hydrogel into the anterior chamber is effective for modeling COH, can respond to most commonly used hypotensive drugs, and is non-toxic to the eyes.

MeSH Keywords: **Evaluation Studies • Hydrogel • Models, Animal**

Full-text PDF: <https://www.medscimonit.com/abstract/index/idArt/925852>

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Background

Glaucoma is a neurodegenerative disease that is irreversible and can cause blindness. It is often characterized by a progressive loss of retinal ganglion cells (RGCs). There are approximately 64.3 million glaucoma patients in the 40- to 80-year-old age group globally, and this is expected to reach 76 million and 111.8 million by 2020 and 2040, respectively [1].

At present, many methods have been attempted for establishing an animal chronic ocular hypertension (COH) model to reflect the basic characteristics of glaucoma. These include gene mutation [2–4], laser photocoagulation on the aqueous humor outflow pathway [5], injection of hypertonic saline into the episcleral vein [6,7], cauterization or ligation of episcleral veins [8,9], infusion of microbeads into the anterior chamber [10,11], and injection of hyaluronic acid [12,13] or cross-linked hydrogel [14,15] into the anterior chamber. Although these methods can to some extent simulate the glaucomatous optic neuropathy caused by chronic high intraocular pressure (IOP), their evaluation is still limited to increased IOP and loss of RGCs. Manifestations such as the impairment of visual function and the reaction to ocular hypotensive drugs are often overlooked, along with the safety of modeling methods. Unilateral evaluation methods reduce the reliability of the model, and the lack of security considerations present hidden dangers to its application. Therefore, we hope to propose a more comprehensive evaluation for the COH model.

We previously presented a COH-inducing model using a single intracameral injection of cross-linking hydrogel in rats, and observed the COH modeling success rate, the amplitude of IOP rise, and the duration [16]. The changes in retinal thickness, loss of RGCs, and the toxic effect of cross-linked hydrogel on RGCs were also evaluated [16]. To supplement the evaluation of other glaucoma characteristics and safety of our COH model, this study assessed the damage to visual function, the response to 5 common ocular hypotensive drugs (brimonidine, timolol, brinzolamide, pilocarpine, and bimatoprost), and the toxic effect of hydrogel injection on the structure of the anterior chamber and corneal endothelial cells (CECs). This can provide a more complete glaucoma model evaluation for use in glaucoma basic research.

Material and Methods

Animals

Animal experiments were approved by the Institutional Review Board of Ruijin Hospital, Shanghai, China and followed the guidelines of the Association for Research in Vision and Ophthalmology (ARVO). All animals were purchased from Zhejiang Vital River Laboratory Animal Technology Co., Ltd (Zhejiang, China). Rats were all Sprague-Dawley (SD) adult males, weighing approximately 250 g, and fed under a 12-h light-dark cycle. The numbers of rats used in each procedure are listed in Table 1.

Cross-linking hydrogel

The HyStem Cell Culture Scaffold kit (HCCS; Sigma-Aldrich, St. Louis, MO) containing Extralink (a thiol-reactive polyethylene glycol diacrylate) and HyStem (a thiol-modified carboxymethyl hyaluronic acid) was used.

Ocular hypotensive drugs

The ocular hypotensive drugs tested were brimonidine (0.2% brimonidine tartrate, Allergan Pharmaceuticals, Dublin, Ireland), timolol (0.5% timolol maleate, Bausch & Lomb, Tampa, FL), brinzolamide (1% brinzolamide, Alcon, Fort Worth, TX), pilocarpine (0.5% pilocarpine nitrate, Bausch & Lomb, Tampa, FL), and bimatoprost (0.03% bimatoprost, Allergan Pharmaceuticals, Dublin, Ireland).

Instruments

We used the TonoLab Rebound Tonometer (Icare, Vantaa, Finland), Ophthalmic Surgery Microscope System (Model: OMS-800 Standard; TOPCON, Tokyo, Japan), and Multi-focal Visual Diagnostic Test System (Model: LKC-UTAS-SBMF; LKC Technologies, Gaithersburg, MD).

IOP elevation induction

COH was induced in the right eyes of rats based on the descriptions of our previous study [16]. Briefly, intraperitoneal injections of ketamine hydrochloride (25 mg/kg; Sigma-Aldrich)

Table 1. Number of animals used in separate procedures (CECs sourced from the same eyes).

Procedure	Number	Procedure	Number
Flash Visual evoked potentials (F-VEP)	112	Histological analysis of anterior segment	24
Ocular hypotensive drugs administration	30	Purification of corneal endothelial cells (CECs)	24
CCK-8 assay of CECs	24	TUNEL assay of CECs	24

and xylazine (10 mg/kg; Sigma-Aldrich) were used to anesthetize the rats, and topical 0.5% proparacaine hydrochloride (Bausch & Lomb) were applied to the corneal surface. Elevation of IOP was achieved through injecting 7 μ L HCCS consisting of HyStem and Extralink at a ratio of 4: 1 into the anterior chamber with a 31-gauge insulin syringe (BD Ultra-Fine, America). Due to risks of potential inflammation caused by contralateral COH eyes, the left eyes were not designated as controls [17]. Instead, an equal amount of phosphate-buffered saline (PBS) was injected into the right eyes of the control group. We used 0.3% Ofloxacin Eye Ointment (Santen Pharmaceutical, Osaka, Japan) to prevent infections.

IOP measurement

IOP was measured using a TonoLab Rebound Tonometer (Icare, Vantaa, Finland). Brief systemic anesthesia through isoflurane inhalation (2–4%; Sigma-Aldrich) was administered to minimize variations of IOP induced by stress and movements. To mitigate bias caused by circadian rhythm, measurements were strictly conducted between 10 A.M. and 2 P.M. The average readings were based on 6 consecutive measurements and analyzed using TonoLab's internal program.

Flash Visual evoked potentials (F-VEP)

VEPs were recorded subdermally via needle electrodes (Multifocal Visual Diagnostic Test System; LKC Technologies). Rats were under ether anesthesia throughout the recordings. The collecting electrode, which recorded flash VEP, was placed at 3 mm before the tip of the lambdoidal suture to receive flash stimuli, while the reference electrode was placed at the center of anterior fontanelle, and the pin electrode was placed on the tail, acting as the grounding electrode. With the contralateral eye covered by an opaque black eyeshade, flash stimuli from a visual electrophysiological system were performed on one eye, and the VEP response was recorded at the contralateral visual cortex 3 times. Repetition frequency of flash stimulus was adjusted to 2.0 Hz, and light intensity of flash stimulus at 0 dB ($3.0 \text{ cd}\cdot\text{s}\cdot\text{m}^{-2}$). The response duration was 300 ms. VEPs were obtained from right eyes by averaging 80 responses. The latency (ms) and amplitude (μ V) of the P1 wave were observed, and each eye was measured 3 times to obtain an average value.

Drug administration

Five commonly used ocular hypotensive drugs were selected for this study: brimonidine (2 receptor agonist), timolol (receptor blocker), brinzolamide (carbonic anhydrase inhibitor), pilocarpine (cholinergic agent), and bimatoprost (prostaglandin analogues). Fourteen days after COH were induced, rat eyes that developed significant elevation of IOP were topically treated

with test drugs. For single-dose studies, a single dose (15 μ L) of either the vehicle control (0.9% saline) or one of the 5 ocular hypotensive drugs was applied to each right eye with a pipette. IOP was measured before and 2, 4, 6, 8, and 24 h after administration of the drug or vehicle control. For repeated-dosing studies, rats received eye drops of either the vehicle control or one of the 5 ocular hypotensive drugs once a day consecutively for up to 1 week. IOP was measured before and 2 h after each administration of these eye drops, and the average of the 2 measurements was recorded as daily IOP value.

Histological analysis of anterior segment

After the rat eyes were transcardially perfused with ice-cold saline and then 4% PFA, then they were enucleated and fixed in 4% PFA at 4°C for 24 h. The eyes then were bisected, and the lens, vitreous, and retina were removed to preserve the anterior segment. After being washed in phosphate-buffered saline (PBS), the eyecups were immersed in 30% sucrose solution for 4 h at 4°C before being frozen in optimum cutting temperature compound (OCT; Sakura Finetek, Japan). Cryosections of 10 μ m thickness were cut in an orientation parallel to the center of the pupil using a cryostat (Model: CM3050S; Leica Biosystems, Buffalo Grove, IL) and collected on Superfrost Plus glasses (Thermo Fisher Scientific, Waltham, MA). The slices were permeated with cold 0.3% Triton X-100 solution (30 min) and blocked with 1% bovine serum albumin (1 h) at room temperature. Then, the slices were incubated with a terminal deoxynucleotidyl transferase-mediated deoxyuridine triphosphate nick-end labeling (TUNEL) kit (In Situ Cell Death Detection Kit; Roche Diagnostics Corporation, Indianapolis, IN) and further incubated with 4',6-diamidino-2-phenylindole (DAPI; Sigma-Aldrich) for 5 min. The sections were then cover slipped with the fade-retardant mounting medium (Thermo Fisher Scientific) and examined under microscopy (Carl Zeiss Microscopy).

Purification of CECs

A total of 48 eyeballs of adult SD rats (weighing 200–250 g) were removed aseptically, and the corneas were separated and placed in a sterile petri dish. The epithelial cells were scraped off the corneas, and the residual epithelial cells were removed by further wiping with sterile gauze. The processed corneas were rinsed 3 times with Dulbecco's phosphate-buffered saline (D-PBS) and cut into pieces of approximately 1×2 mm. The corneal pieces were placed in a 24-well tissue culture plate with the endothelium facing down in culture solution, and then were placed in a 37°C incubator. The pieces were removed gently after the CECs had grown. The CECs were collected and co-cultured with the cross-linking hydrogel for 1, 3, and 7 days, and then were assessed by TUNEL assay and CCK assay as described in the paragraphs below.

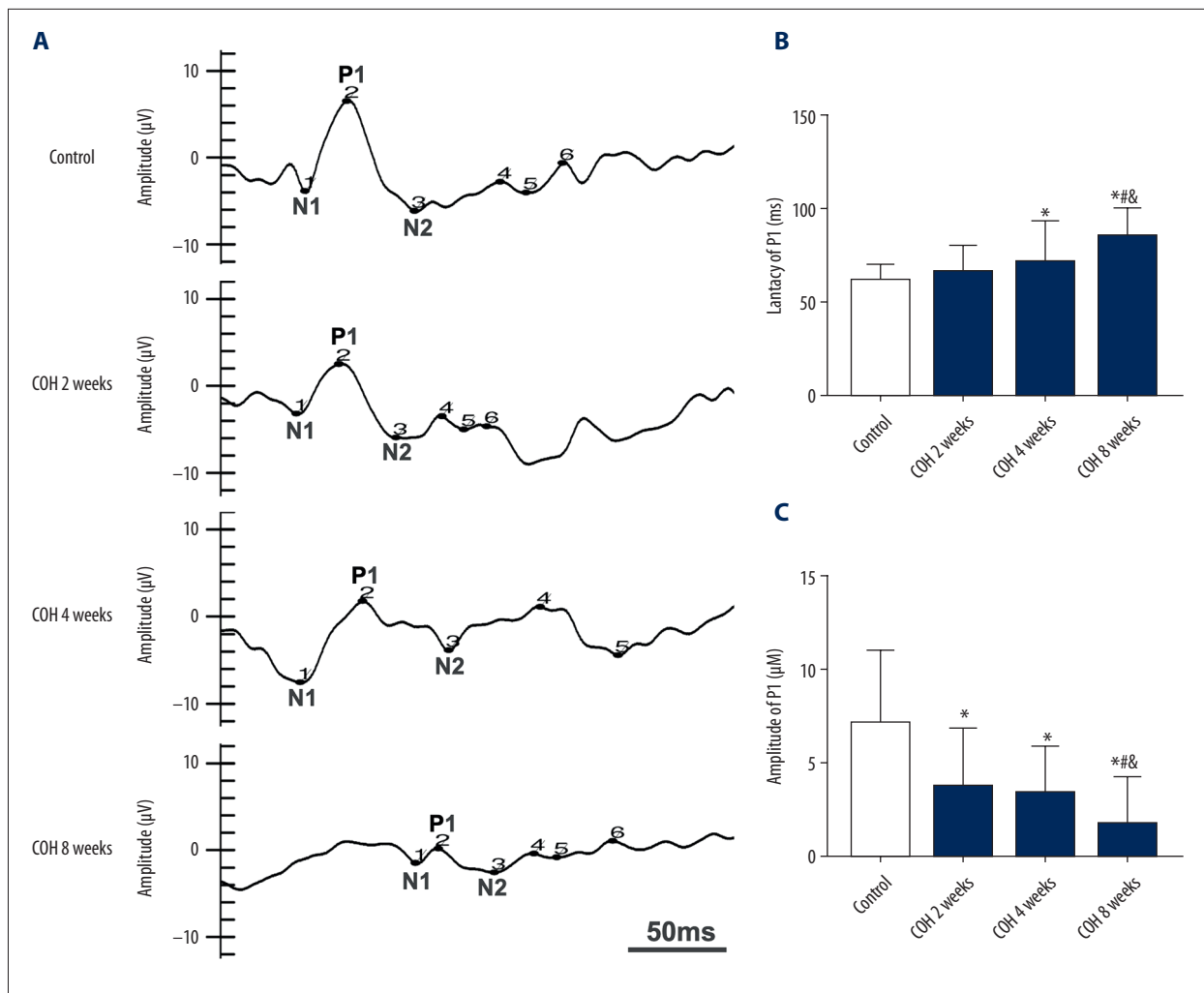


Figure 1. Changes of latency and amplitude of P1 induced by chronic ocular hypertension (COH) in Flash Visual evoked potentials (F-VEP) test. **(A)** F-VEP detection waveforms of rats at 0, 2, 4, and 8 weeks of COH. **(B)** The latency of the P1 increases along in accordance with the prolongation of COH. The latencies of 4 and 8 weeks after COH were significantly longer than that of the control group ($n=28$; * $P<0.05$ versus the control group, # $P<0.05$ versus the COH 2 weeks group, & $P<0.05$ versus the COH 4 weeks group); **(C)** The amplitude of P1 decreased along with the prolongation of COH. The amplitudes at 2, 4, and 8 weeks after COH were significantly lower than that of the control group ($n=28$; * $P<0.05$ versus the control group, # $P<0.05$ versus the COH 2 weeks group, & $P<0.05$ versus the COH 4 weeks group).

Cellular TUNEL assay

Cellular TUNEL assay was performed as previously described [16]. Briefly, purified CECs were seeded in 24-well plates with 1 mL of approximately 5×10^5 cells in each well and cultured for 24 h. Hydrogel were added to the culture wells at a 1: 10 volume ratio. After culturing for another 1, 3, and 7 days, the nuclear DNA fragmentations of apoptotic cells were assayed through TUNEL method using the In Situ Cell Death Detection Kit (Roche Diagnostics Corporation). Total cell number and TUNEL-positive cells were counted in 6 randomly selected fields of each coverslip and averaged to identify the proportion of TUNEL-positive cells.

CCK-8 assay

CCK-8 assay was performed as previously described [16]. Briefly, purified CECs were seeded in 96-well plates with 100 µL of approximately 5×10^3 cells in each well and cultured for 24 h. Hydrogel was added to the culture wells at a 1: 10 volume ratio. After culturing for another 1, 3, and 7 days, cytotoxicity was assayed by the Cell Counting Kit-8 (CCK-8; Sigma-Aldrich) following the manufacturer's instructions. The optical density (OD) value of absorbance at 450 nm was then measured using Fluoroskan Ascent FL (Thermo Fisher Scientific), which reflected the activity of cell dehydrogenase and the number of living cells.

Table 2. Summary of Flash Visual evoked potentials (F-VEP) test.

Groups	N	Latency (ms)	Amplitude (μ V)	P1	P2
Control	6	62.23 \pm 1.65	7.14 \pm 0.73		
COH 2 weeks	6	67.06 \pm 3.39	3.64 \pm 0.85	0.16	0.005
COH 4 weeks	6	73.02 \pm 4.31	3.25 \pm 0.49	0.017	<0.001
COH 8 weeks	6	85.77 \pm 3.16	1.67 \pm 0.50	<0.001	<0.001

P1=P value for the Chronic ocular hypertension (COH) 2/4/8 weeks – Control comparison group in latency; P2=P value for the COH 2/4/8 weeks – Control comparison group in amplitude.

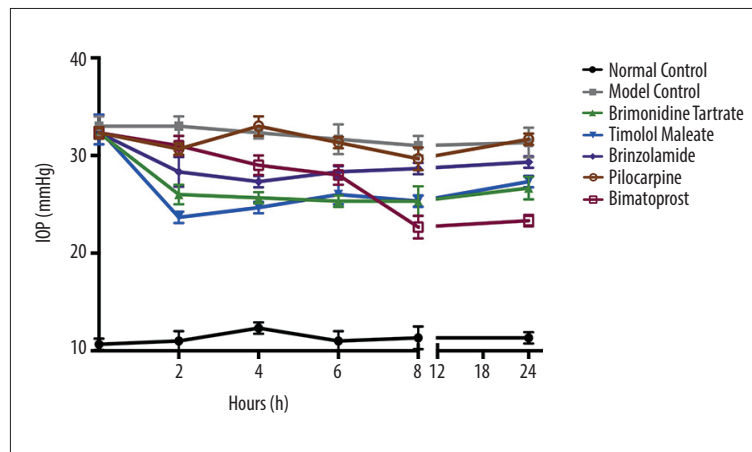


Figure 2. Changes of intraocular pressure (IOP) after a first administration of ocular hypotensive agents. The results showed that brimonidine, timolol, brinzolamide, and bimatoprost eye drops had an ocular hypotensive effect on rats with elevated IOP. IOP of brimonidine-treated and timolol-treated groups was reduced to a minimum level at 2 h after administration, and then slightly rebounding, but was still significantly lower than that of the model control group at 24 h after administration ($P<0.05$). The effect of bimatoprost was not obvious within 6 h after administration. The IOP was reduced at 8 h after administration and maintained at that level until 24 h. The IOP of the rats treated with brinzolamide was reduced to the lowest at 4 h after administration, and then gradually increased. The IOP at 6 h after administration was not significantly different from that of the control group ($P>0.05$). IOP returned to pre-dose levels at 8 h after administration. The ocular hypotensive effect of pilocarpine in this model was not obvious.

Statistical analysis

All data are expressed as mean \pm standard deviation. Statistical analyses were performed with SPSS software (IBM SPSS statistics Version 19.0; IBM, Armonk, NY). Statistical differences between groups were estimated using the *t* test or one-way ANOVA.

Results

Visual function impaired following IOP elevation

Typical NPN waveforms were recorded in each group. The waveforms of the rats undergoing 8 weeks of COH were slightly irregular when compared with the other groups. These waveforms are named according to the international clinical visual electrophysiology regulations. The first negative wave N1 appears

at the beginning of the waveform, and the positive wave appearing immediately after is the P1 wave. The negative wave following P1 is the N2 wave. Following the prolongation of IOP elevation time, P1 latency of 4 and 8 weeks after COH was significantly longer than in the control group. Meanwhile, the P1 amplitude at 2, 4, and 8 weeks after COH was significantly lower than in the control group (Figure 1). The results indicated that the COH model could effectively cause optic nerve function impairment (Table 2).

IOP-lowering effects by ocular hypotensive drugs intervention

Five ocular hypotensive drugs (brimonidine, timolol, brinzolamide, pilocarpine, and bimatoprost) were used in this study. The IOP was monitored 2, 4, 6, 8, and 24 h after the first use of the hypotensive drug (Figure 2). Administration was conducted once a day for the next 6 days, and IOP was monitored before

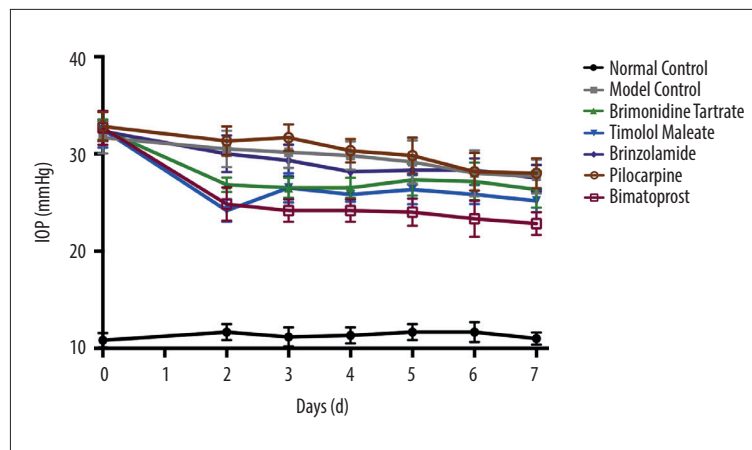


Figure 3. Intraocular pressure (IOP) changes of the rats administered continuously for 1 week. IOP of rats treated with brimonidine, timolol, and bimatoprost were significantly lower than in the control group at 2–7 days after administration, and the difference was statistically significant ($P < 0.05$). The IOP of the rats treated with brinzolamide and pilocarpine were not significantly different from the control group at 2–7 days after administration ($P > 0.05$).

and 2 h after administration (Figure 3). The results showed that brimonidine, timolol, brinzolamide, and bimatoprost eye drops were effective on rats with elevated IOP. Among them, timolol has the fastest effect, while bimatoprost has the longest effect. The hypotensive effect of pilocarpine was not obvious. The results indicated that the COH model responded well to most of the ocular hypotensive drugs, being consistent with the characteristics of glaucoma.

Morphological and apoptotic changes of anterior segment following IOP elevation

At 2, 4, and 8 weeks after induction of COH, the morphological changes of the cornea, iris, ciliary body, and anterior chamber angle were observed in frozen sections of rat anterior segments. TUNEL staining was used to detect the impact of cross-linking hydrogel on the apoptosis of CECs after anterior chamber injection. The results showed that no significant morphological change was observed in the anterior segment of COH group, and the apoptosis of CECs were not significantly affected by anterior chamber injection of cross-linking hydrogel (Figure 4).

Cell death assays indicates no cytotoxicity of hydrogel on CECs

To determine whether there is cytotoxicity of hydrogel on CECs, CCK-8 assay was used to evaluate cell survival referencing their varying degrees of exposure to hydrogel. The OD values increased gradually with the prolongation of culture time; however, co-culture with hydrogel showed no significant effects on the OD values (Table 3). The cross-linking hydrogel's biocompatibility was further evaluated using TUNEL assay (Table 4). As the culture time was prolonged, the proportion of TUNEL-positive CECs increased gradually in the hydrogel co-culture

group and the control group, while statistical analysis revealed that the culture time had significant effects on the increase in proportion of TUNEL-positive cells. However, co-culture with hydrogel did not lead to significant changes. These results suggest that cross-linking hydrogel has a high cytocompatibility with CECs and lacks cytotoxicity for CECs (Figure 5).

Discussion

COH animal models are prerequisites in investigating the mechanisms and treatment for glaucomatous optic neuropathy. In a previous study, we established a COH model induced through a single injection of hydrogel into the anterior chamber, and conducted supplementary evaluations on the performance and safety of the model in a recent study. The results show the model can simulate structural and functional changes of glaucoma and responds well to most ocular hypotensive drugs. The cross-linking hydrogel had no obvious toxic effect on the anterior segment and corneal endothelial CECs.

To date, a range of COH models have been reported. Rodents, including rats and mice, have a high degree of conservation with the human genome and can be genetically manipulated at the genome level. They share similar anatomical [18] and developmental [19] characteristics of the anterior chamber, especially in the aqueous outflow pathway, with humans. In addition, they are inexpensive, easy to handle and obtain samples from, and can reproduce in large numbers according to experimental needs. These advantages make the rodent animal models the most common models used for glaucoma research. Various COH models have been established in rodents. In addition to the well-known and widely used methods, like gene mutation [20], other methods have become important,

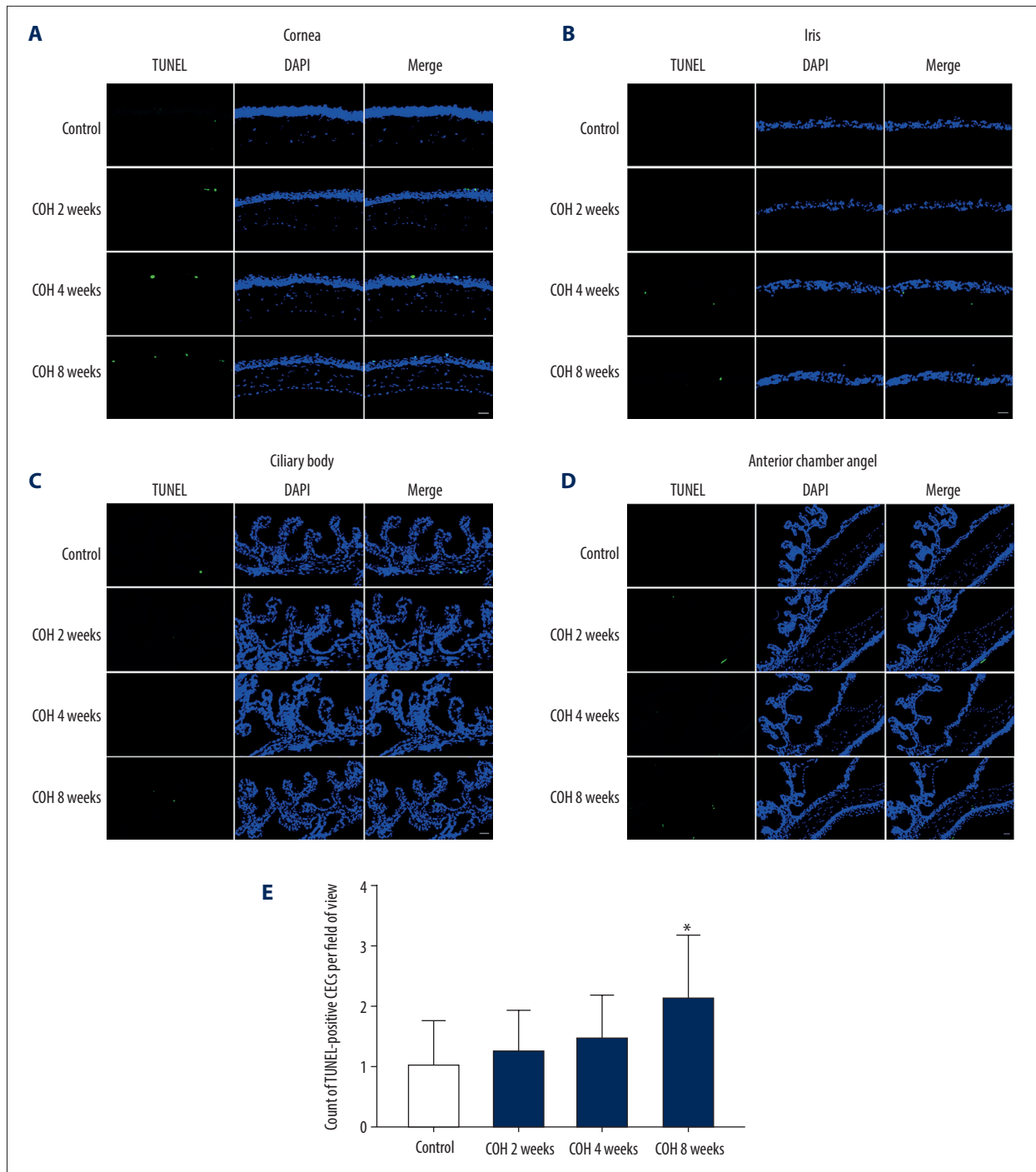


Figure 4. Histomorphological and apoptotic changes of cornea, iris, ciliary body, and anterior chamber induced by chronic ocular hypertension (COH). **(A–D)** The frozen sections and TUNEL staining of the anterior segment (**A–C** represent cornea, iris, and ciliary body, respectively; magnification 200, scale bar=100 μ m); **(D)** Anterior chamber angle, magnification 100, scale bar=100 μ m). **(E)** There was no significant difference in the number of TUNEL-positive corneal endothelial cells (CECs) in the corneas at 2 and 4 weeks after ocular hypertension compared with that of the control group (n=6, P=0.503, 0.207, respectively). At 8 weeks after ocular hypertension, the number of CECs positive for TUNEL staining increased in a single field of view compared with the control group.

Table 3. Summary of CCK-8 assay result.

Time	OD values of CCK-8		Statistical significance between groups (n=6)
	Control	Hydrogel co-culture	
1 day	0.45±0.01	0.36±0.01	P=0.69
3 days	1.46±0.09	1.22±0.08	P=0.08
7 days	1.75±0.03	1.68±0.04	P=0.19

Table 4. Summary of TUNEL assay result.

Time	TUNEL-positive proportions (%)		Statistical significance between groups (n=6)
	Control	Hydrogel co-culture	
1 day	2.29±0.36	2.52±0.53	P=0.73
3 days	4.07±1.23	3.83±0.38	P=0.54
7 days	7.06±1.41	7.96±1.34	P=0.12

such as laser photocoagulation of the trabecular meshwork at the limbus [21,22], episcleral veins injection of hypertonic saline [6,7], occlusion of the episcleral veins by cauterization or suture ligation [9,23], and injection of microbead [11,24,25] or hyaluronic acid into the anterior chamber [13]. Other new rodent COH models, such as circumlimbal suture [26] and intracameral injection of conjunctival fibroblasts [27], have also been reported in recent years. However, various challenges also exist with the currently available rodent models. As a representative of the spontaneous high IOP model, DBA/2J mice develop a genetically determined form of pigmentary glaucoma that is related to recessive mutations in 2 genes, glycosylated protein nmb (Gpnmb) and tyrosinase-related protein 1 (Tyrrp1) 3–5. However, the incidence of qualified spontaneous high IOP and the time and cost required to obtain a pure transgenic line are not satisfactory, thereby limiting its wide application. Regarding laser photocoagulation, the IOP elevation induced by this approach is variable and extra treatments are often needed to maintain ocular hypertension, which may pose a risk of ocular complications [28,29]. Injection of hypertonic saline into the episcleral veins requires special micro-injector equipment that is difficult to operate. The uneven IOP rise further requires a large sample size to compensate for error [6,7]. For the COH models with episcleral vein cauterization or ligation, there may be surgery-associated risk of creating venous drainage of the choroid. Furthermore, hypoxia-mediated changes that cause confounding effects regarding the retina and neovascularization of episcleral vessels may compensate for the drainage of aqueous humor, which will reduce the reliability of the model [30–32]. The COH model induced by intracameral injection of microbeads or hyaluronic usually requires repeated injections, which brings a high risk of cornea decompensation and intraocular hemorrhage [10,13,33,34]. In

addition, the presence of residual microbeads in the retina is likely to affect observation and counting of RGCs [10,33]. To address these issues, Zhao et al. [26] reported a circumlimbal suture approach to induce chronic IOP elevation in mice with 5–6 subconjunctival anchor points behind the limbus. This approach avoided introducing foreign materials into the eye, yet the rate of ocular complications was high (71% of eyes showed developed hyphema and 29% showed suture breakage, slippage, or conjunctival tear), with a success rate of only around 50% [26]. Recently, a novel approach to reliably induce chronic IOP elevation in rats using injection of conjunctival fibroblasts was introduced by Ayumi and colleagues [27]. The authors were inspired by the clinical occurrence of epithelial downgrowth into the anterior chamber, which is often observed during invasive ocular surgery with a wide corneal incision. In the present study, rat conjunctival fibroblasts were injected into rats' anterior chambers to induce an increase IOP, which lasted for 28 days. Although a sustained increase in IOP was achieved at a high success rate, there were still several limitations to our study. First, the analysis of the duration of elevated IOP was relatively short. Second, the fluctuation of IOP values was intense. Third, the view of the posterior chamber of the eye was continuously obstructed by cell accumulation and iris adhesion on the anterior surface of the lens. Therefore, this method needs to be further improved. In addition, the current evaluation method for the COH model is too simple and one-sided. Most of the proposed models have only been evaluated for increase of IOP and loss of RGCs, and changes in retinal thickness and visual impairment are rarely mentioned. The safety of modeling methods is of less concern.

Taking the aforementioned limitations into account, it was necessary to explore a stable, effective, and simple COH animal

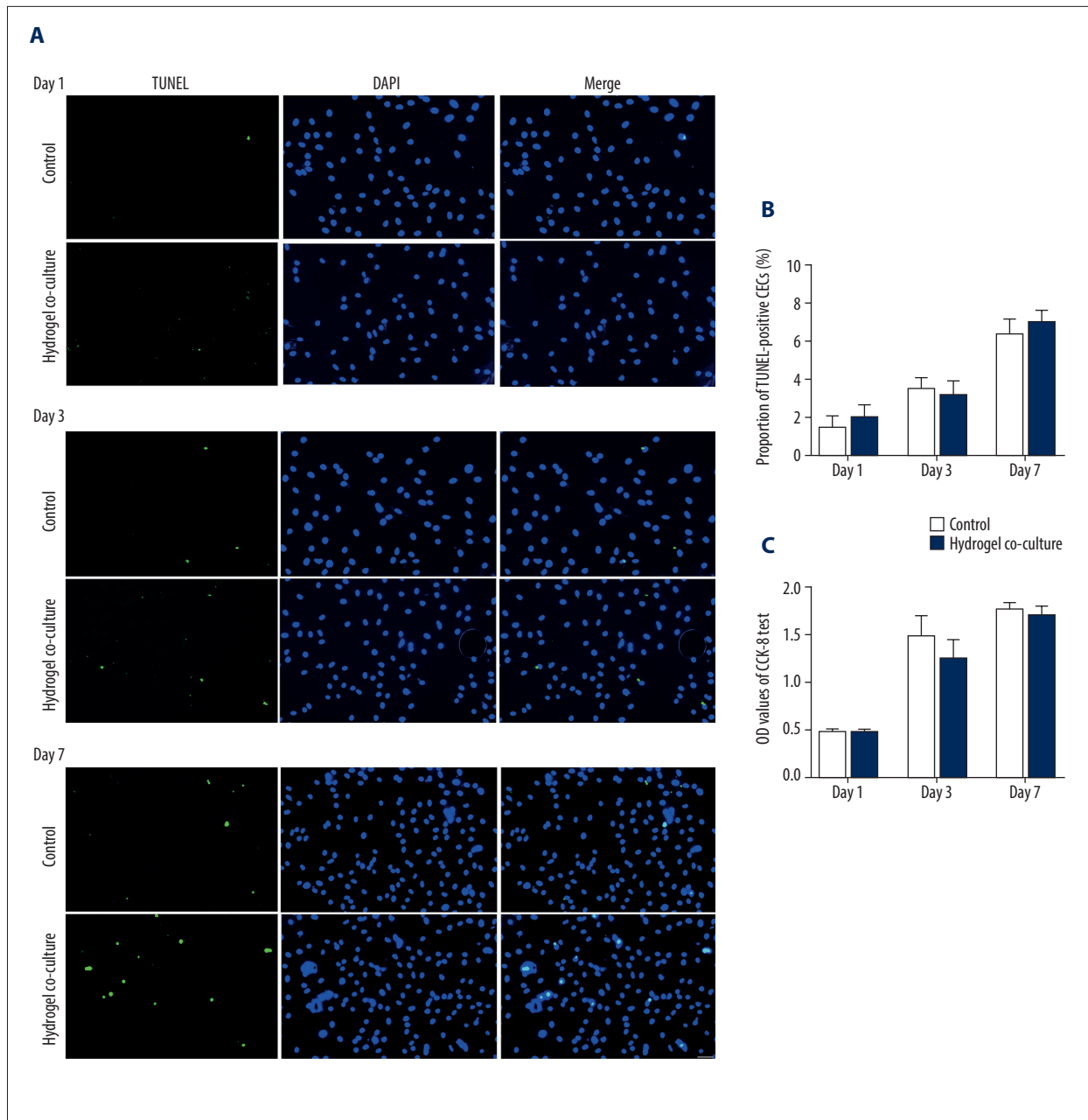


Figure 5. Effect of cross-linking hydrogel on apoptosis and survival of corneal endothelial cells (CECs). (A) The isolated and purified CECs were co-cultured with cross-linking hydrogel for 1, 3, and 7 days, and the apoptosis of CECs was detected by TUNEL staining (magnification 200, scale bar=100 μ m); (B) The proportion of CECs positive for TUNEL staining in the control group and the cross-linking hydrogel co-culture group at each time point; (C) The OD values of CCK-8 assay in the control group and the co-culture group at various time points.

model and to conduct a comprehensive evaluation. The cross-linking hydrogel has low toxicity, high water-retention capacity, multifunctional usefulness, and excellent biocompatibility [35,36], and has been applied in several biomedical research fields such as drug release [37,38], cell culture [39], tissue regeneration [40–42], 3D bioprinting [43], and prevention of postoperative intra-abdominal adhesion [44]. In the field of

ophthalmology, cross-linking hydrogel has been used in tissue engineering, including corneal transplant [45,46] and retinal progenitor cell transplantation [47], drug delivery systems for multiple eye diseases such as glaucoma [48] and uveitis treatment [49], treatment of ocular surface [50] and lacrimal diseases [51], and substitution of the vitreous body [52,53], showing great potential. It was reported that a hydrogel based on

thiol-modified derivatives of hyaluronic acid can become gelled at room temperature and be modulated in both gel compliance and gelation time by adjusting the ratio of its components [54,55]. Due to the above advantages of cross-linking hydrogel, we chose to inject it into the anterior chamber to induce COH. The IOP elevation of the model was stable and sustained even with only 1 injection, and this will help reduce possibilities of infection and also provide sufficient time window for studying pathological changes in this chronic disease. Therefore, cross-linked hydrogels have seen increased use in the construction of glaucoma models in recent years [14,15].

The results of our previous evaluation showed that 32 of the 50 eyes that received hydrogel anterior chamber injection had elevated IOP (average, 23.6 ± 3.8 mmHg), which was sustained for more than 10 weeks, significantly longer than in the control group (average, 14.4 ± 1.2 mmHg, $n=10$). The COH model can simulate structural changes of glaucoma (including the loss of RGCs and morphological changes of retina). In addition, the cross-linking hydrogel has no toxic effects on RGCs [16]. In this evaluation, we focused on the evoked electrophysiological signals extracted from the electroencephalographic activity in the visual cortex, which were recorded from the overlying scalp, which is important for the evaluation of visual function. The present study used F-VEP to detect the visual function following COH induction. Significantly longer latencies and smaller amplitudes of P1 in the hydrogel-injected eyes, in comparison with the fellow control eyes, support that IOP elevation following intracameral injection of hydrogel effectively impaired visual function of the rats, which is consistent with the report of Liu et al. [14]. The F-VEP waveforms of COH eyes were similar to that of glaucoma patients in seen in clinical practice, which indicated that the COH model can induce visual impairment similar to that of human glaucoma. These results suggest that our model effectively simulates pathological changes that occur in human glaucoma, and that intracameral injection of cross-linking hydrogel has potential for further application in serial studies of RGC-associated degeneration, in both structural and functional terms.

Following appropriate intracameral injection, the cross-linking hydrogel blocks the angle of the anterior chamber, then obstructs the pathway of aqueous outflow. The COH model could be used for the preclinical evaluation of effectiveness of aqueous humor production suppressants. Our results indicated that aqueous production suppressants, such as brimonidine, timolol, and brinzolamide, had a superior IOP-lowering effect on hydrogel-induced COH. The 3 drugs exhibited patterns of IOP-lowering kinetics similar to those seen in humans and monkeys [56]. Interestingly, for the drugs that increased the outflow of aqueous humor, topical application of bimatoprost

lowered the elevated IOP, while topical application of pilocarpine showed no significant effect on the IOP in the eyes with hydrogel-induced COH. This discovery was the opposite of that observed in a microbead-induced COH model by Yang et al. [57]. We speculated that the hydrogel blocked the angle of the anterior chamber to affect the outflow of aqueous humor, which restricted the hypotensive effect of pilocarpine, while bimatoprost reduced IOP by promoting the outflow of aqueous humor from the uveoscleral pathways. The IOP-lowering effect of timolol was short-lived, which was consistent with the reported fast clearance of the drug in human patients. Brimonidine could reduce IOP to a minimum level 2 h after administration, while brinzolamide needed 4 h to achieve this effect. The lowering of IOP introduced by bimatoprost took effect 6 h after administration and lasted until 24 h later, and the results were consistent with those reported in human patients [58]. These results suggest that rat eyes have receptors and pathways capable of responding to these pharmaceutical substances. In summary, a single intracameral injection of cross-linking hydrogel successfully induced a COH model in rats, and the disease characteristics of the model were consistent with clinical observations in humans.

In the biosafety assessment of hydrogels, in addition to the toxicity test on RGCs in the previous study, we also studied the effects of hydrogel injection on the structure of the anterior chamber and the toxic effects on CECs. The results showed there was no significant change in the anterior segment in the COH group, and the apoptotic status of CECs was not obviously affected by the coexistence with the cross-linking hydrogel. It was also found that the intracameral injection of cross-linking hydrogel had no significant toxic effect on the angle structure of the anterior chamber and did not play a role in proliferation and apoptosis of CECs, which allowed us to use hydrogels more confidently when modeling.

Conclusions

A single intracameral injection of cross-linking hydrogel can successfully establish the COH model in rats. The model can simulate structural and functional changes of glaucoma and respond well to most ocular hypotensive drugs. The cross-linking hydrogel had no obvious toxic effect on anterior segment, CECs, and RGCs. To conclude, a single intracameral injection of cross-linking hydrogel can provide a stable, effective, and simple COH model for use in basic research on glaucoma. We also advocate the establishment of a more comprehensive evaluation system for a COH model to ensure the reliability of its performance and the safety of modeling methods.

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