What is the impact of intravitreal injection of conbercept on neovascular glaucoma patients: a prospective, interventional case series study

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Research Article

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Abstract

Background: The aim of the present study was to evaluate the efficacy and safety of intravitreal conbercept combined with trabeculectomy and panretinal photocoagulation for neovascular glaucoma (NVG).

Methods: Fifty patients (54 eyes) with NVG were included in this prospective study. Fifty-two eyes initially underwent intravitreal conbercept, and then trabeculectomy and panretinal photocoagulation were performed. Preoperative and postoperative best-corrected visual acuity (BCVA), intraocular pressure (IOP), number of antiglaucoma medications, and surgical complications were recorded. The levels of VEGF-A, TGF-β1 and PLGF in aqueous humour that were collected during surgery were measured by enzyme-linked immunoabsorbent assay (ELISA). Light microscopy and transmission electron microscopy were used to observe the surgically excised trabecular tissue; enucleation was performed for 2 eyes, and light microscopy was used as the histopathological control.

Results: At the last follow-up visit, mean IOP was reduced from 48.1±14.2 mmHg to 23.2±8.7 mmHg, and the mean number of antiglaucoma medications used decreased from 3.0 (3.0, 4.0) to 1.0 (0.0, 1.0) (both $P < 0.05$). After patients underwent intravitreal injection, the concentrations of VEGF-A and TGF-β1 in aqueous humour in NVG patients decreased from 168.8±13.4 pg/ml and 159.6±15.4 pg/ml to 160.2±7.6 pg/ml and 151.9±2.3 pg/ml, respectively (both $P < 0.05$). Light microscopy revealed neovascularization regression in the iris in specimens with intravitreal conbercept. Electron microscopy revealed endothelial cell degeneration in the conbercept-treated specimens.

Conclusions: Our initial findings suggest that intravitreal conbercept is an effective treatment for managing neovascular glaucoma, with fewer postoperative complications in the short-term.

Trial registration: Current Controlled Trials ChiCTR1800019918, 8 December 2018, Retrospectively registered.

Keywords: Neovascular glaucoma; conbercept; anti-vascular endothelial growth factor; trabeculectomy

Background

Neovascular glaucoma (NVG) is a refractory glaucoma that caused by central retinal vein occlusion (CRVO), diabetic retinopathy (DR), and ocular ischaemic syndrome. The common clinical manifestation of NVG is iris and anterior chamber angle neovascularization due to retinal ischaemia [1]. The main treatment of NVG are control intraocular pressure (IOP) and treat primary disease. Anti-glaucoma drugs or panretinal photocoagulation (PRP) appeared to be an effective option for rubeosis iridis and open-angle glaucoma stage. But in the late stages of NVG, proliferative fibrovascular membrane causes angle closure so that IOP sharp increases. In this stage, glaucoma filtering surgery for NVG is effective, but the success rate is poor because of iris and angle neovascularization which causes hyphema, filtering bled scarring and so on.

Vascular endothelial growth factor (VEGF) is synthesized because of retinal ischaemia and hypoxia [2].PRP can not rapidly regress iris and angle neovascularization, and PRP is difficult to perform with media opacities due to high IOP. Intravitreal anti-VEGF agents have been confirmed to decrease the concentration of VEGF in the aqueous humour and regress iris neovascularization. Therefore, the use of anti-VEGF agents before trabeculectomy can reduce intraoperative and postoperative complications [2-4].PRP can improve retinal ischemia status which reduce the release of VEGF. According to previous studies, intravitreal injection of anti-VEGF agents such as bevacizumab and ranibizumab combined with glaucoma filtering surgery and PRP has been shown that could to regress iris neovascularization rapidly and effectively reduce IOP [3, 4].

Recently, a new anti-VEGF agent, conbercept (KH902, Chengdu Kang Hong biotech Co., Sichuan, Chengdu), has shown that quickly regress choroidal neovascularization for age-related macular disease clinically, but the effect of conbercept for iris neovascularization is unknown. This study was performed to assess the efficacy and safety of conbercept for the treatment of NVG in 54 eyes of 50 NVG patients and to explore the relationship between VEGF-A, transforming growth factor-β1 (TGF-β1), placenta growth factor (PLGF) and NVG, which may be a new target for NVG.

Methods

We performed a study of a consecutive series of 54 eyes of 50 patients who were diagnosed with NVG in Tianjin Eye Hospital from May 2015 to March 2017. Fifty-two eyes of 48 patients received intravitreal injection, including 37 males (40 eyes) and 11 females (12 eyes) ranging in age from 18 to 82 years (55.9 + 14.7). Twenty-three eyes had DR, 23 eyes had CRVO, 5 eyes had ocular ischaemic syndrome and 1 eye had central retinal artery occlusion. The other 2 eyes had absolute glaucoma with unbearable pain, and nucleation was performed to identify iris neovascularization by light microscopy as a control.

The inclusion criteria were (1) NVG patients whose underlying diseases were DR, CRVO, carotid artery stenosis or occlusive disease, (2) patients who did not have PRP before, (3) patients who were 18 to 85 years old, (4) patients who underwent intravitreal injection with a visual acuity ≥ hand motion, or the visual acuity of patients with enucleation had no light perception, (5) patients with an IOP >21 mmHg and under the maximum dose of antiglaucoma medications, (6) patients with iris or angle neovascularization, and (7) patients with or without pupillary pigment epithelium. The exclusion criteria were (1) NVG combined with ocular tumours or uveitis, (2) severe cardiovascular and cerebrovascular diseases, (3) patients who had recent active inflammation of the eye, or (4) patients who failed to complete the scheduled follow-ups for various reasons. The study was carried out with the approval of the ethics committee of Tianjin Eye Hospital (No. TJYYLL-2015-15), and all patients provided voluntary informed consent to participate in the study.

Preoperative indexes included the best corrected visual acuity (BCVA), slit-lamp microscopy examination, gonioscopy examination, IOP, primary disease classification and other measures. BCVA was converted to the logarithm of the minimum angle of resolution (LogMAR), IOP was measured with a Goldmann

tonometer (AT 900, Haag-Strsit AG, Switzerland, Bern), but if patients had corneal oedema due to high IOP, an Icare handheld rebound tonometer (TA01, Finland Aike company, Finland, Helsinki) was used. Iris neovascularization grading was as follows: grade 1 was surface neovascularization of the pupillary zone of the iris involving ≤2 quadrants, grade 2 was surface neovascularization of the pupillary zone of the iris involving ≥2 quadrants, grade 3 was neovascularization of the ciliary zone of the iris involving 1 to 3 quadrants addition to the pupillary zone, and grade 4 was neovascularization of the ciliary zone of the iris involving ≥3 quadrants [5].

All patients were assessed by one glaucoma specialist. Under sterilization and topical anaesthesia (0.5% proparacaine hydrochloride eye drops), anterior chamber paracentesis was performed in 48 patients (52 eyes) with high IOP, and then 0.05 ml conbercept was injected intravitreally via the pars plana, approximately 3.5 mm from the limbus, with a 30G needle. Then, IOP and light perception were examined. Patients were given topical antibiotics and antiglaucoma medications after surgery.

Trabeculectomy was performed when the neovascularization of the iris surface regressed and anterior chamber inflammation was relieved (the interval time was 2 to 7 days). Under peribulbar anaesthesia, a fornix-based conjunctival flap and a half thickness of 4 mm*3 mm square scleral flap was made, a mitomycinC (MMC)(0.4 mg/ml) or 5-fluorouracil (5-FU)(25 mg/ml)-soaked sponge was placed in the scleral flap for 2-3 min before rinsing thoroughly with 30 ml saline. Trabecular meshwork (2 mm*1 mm) was cut, and the peripheral iridectomy was performed. The scleral flap was closed with two 10-0 nylon sutures at its corners, and the conjunctiva was sutured with 10-0 nylon sutures.

Two patients (2 eyes) had enucleation under retrobulbar anaesthesia, the bulbar conjunctiva was cut along the limbus corneae, and then the conjunctiva was separated, the tenon capsule and sclera were bluntly dissected to the equator, the extraocular muscles were cut, the optic nerve and soft tissue were removed, the eye was removed, a foetal eye was implanted in the tenon capsule, the front fascia was closed by pouch suture, the tenon capsule was packed with Vaseline gauze, and the eye socket was placed.

Three weeks or 1 month later, PRP was performed (577 nm yellow light supra scan laser light, Quantel, France, Clermont-Ferrand), which was completed 2-3 times with 250-350 mv energy (appear II-III spot as the standard), an interval time of 0.03 s, a spot diameter of 200 μm, and a total light solidifying point of 1500-2000 points.

The surgically excised trabecular specimens of 26 eyes were immediately immersed in a solution of 10% formalin fixative at room temperature for 24 hours, and then these specimens were dehydrated with different concentrations of alcohol (from 70% to 100%) and made transparent with dimethylbenzene two times. After embedding in paraffin, 5-μm-thick sections were made and allowed to dry overnight. Then, these specimens were stained with haematoxylin-eosin and observed with light microscopy (Leica 400B, Leica company, Germany, Solms).

The iris tissue of the other eyes were immersed in 4% glutaraldehyde and 1% osmic acid solution at 4°C for 4 hours and 1 hour, then these specimens were washed with PBS powder and were dehydrated with gradient acetone. After embedding in 812 resin, these specimens were cut into 60 nm sections and stained with lead citrate and uranyl acetate. Then, transmission electron microscopy (JEM-1230, JEOL company, Japan, Tokyo) was used to observe these sections.

Then, 0.1-0.15 ml aqueous humour samples were collected during intravitreal injection and trabeculectomy and were placed into a sterile Eppendorf tube and rapidly frozen at -80°C for use (all samples were obtained at the beginning of surgery to avoid breakdown of the blood-aqueous barrier that is associated with surgical trauma). The main reagents were human VEGF-A enzyme-linked immunoabsorbent assay (ELISA) kit, human TGF- β1 ELISA kit and human PLGF ELISA kit (China BlueGene Co., China, Shanghai). The concentrations of VEGF-A, TGF- β1 and PLGF in aqueous humour were determined by the double antibody sandwich ELISA method. The standard curve for ELISA (use a four parameter logistic curve fit) was set up, the desired numbers of coated wells were secured in the holder, and then 100 μL of standards or samples were added to the appropriate well, adding 100μL of PBS (pH 7.0-7.2) to the blank control well, adding 50 μL of conjugate to each well (not the blank control well), and covering and incubating the plate for 1 hour at 37°C . The plate was washed five times with diluted wash solution (350-400 μL/well/wash) using an auto washer, and then the plate was dried. Next, 50 μL substrate A and 50 μL substrate B were added to each well, covering and incubating for 15 minutes at 37°C, then adding 50 μL of stop solution to each well, immediately determining the optical density (O.D.) at 450 nm using a microplate reader (ST-360, Shanghai KEHUA Experimental System Co., China, Shanghai).

The postoperative follow-up was 1 year. Patients were followed on schedule (post-injection and 1 day, 1 week, 1 month, 3 months, 6 months, and 1 year after trabeculectomy). BCVA and IOP were recorded. Slit-lamp microscopy examination, gonioscopy examination, the numbers of anti-glaucoma medications, and intraoperative and postoperative complications were recorded. The complete success of the surgery was defined as IOP ≤21 mmHg without any topical ocular hypotensive medication. Partial success was defined as IOP ≤21 mmHg with topical ocular hypotensive medication [6], and surgical failure was defined as IOP >21 mmHg at 2 consecutive follow-up visits even with anti-glaucoma medication or additional glaucoma surgeries, such as filtration surgery and cyclophotocoagulation, or loss of light perception [7].

Statistical analysis was conducted using SPSS 23.0 software (SPSS Inc., America, Chicago). These data on IOP, BCVA and the concentrations of cytokines were confirmed by W test, which was consistent with a normal distribution. Therefore, these results were presented as the mean±standard deviation (SD). The data on the numbers of antiglaucoma drugs were not consistent with a normal distribution. Therefore, the results were expressed as M (Q1, Q3). Single effect repeated-measure analysis of variance and Dunnett's t-test were used to assess differences between BCVA and IOP at different time points. The levels of VEGF-A, TGF- β1 and PLGF were compared by one-way ANOVA and LSD t-tests. The numbers of antiglaucoma medications were compared by K-W tests of multiple groups of independent samples. The correlation

analysis between iris neovascularization and VEGF-A, TGF-β1, PLGF were completed by Spearman rank correlation coefficients. A P values of less than 0.05 were considered statistically significant.

Results

At baseline, iris neovascularization gradings of 25 patients, 17 patients and 10 patients were grade 4, 3 and 2, respectively. Visual acuity remained unchanged in 16 eyes, increased in 16 eyes, and decreased in 7 eyes at the latest follow-up. The IOP in 20 eyes were controlled at 10-21 mmHg, which indicated surgical success; 14 eyes had complete success, and 6 eyes had partial success. Nineteen eyes were surgical failures, and among them, after providing antiglaucoma medicine, massaging the filter bleb and needling the filter bleb with subconjunctival injection, the IOP of 14 eyes who had an IOP>21 mmHg at 2 consecutive follow-up visits decreased to normal. The IOP of 5 eyes who had uncontrolled IOP was controlled by transscleral cyclophotocoagulation (TCP) or trabeculectomy. The data on IOP, BCVA and number of antiglaucoma medications are shown in table 1.

The differences in IOP and BCVA at various of follow-up time points were statistically significant (F=878.486, p=0.000; F=163.157, p=0.000). IOP gradually decreased over time, and IOP was significantly decreased after undergoing trabeculectomy compared to baseline (all p=0.000). BCVA was not significantly increased postoperatively compared to baseline (all p>0.05). There was a statistically significant difference at follow-up time points in the number of antiglaucoma medications (χ^2=294.232, p=0.000), and the number of antiglaucoma medications significantly decreased after undergoing trabeculectomy (all p=0.000) (Table 1).

Hyphema occurred in 9 eyes and were absorbed 2-3 days later. Five eyes underwent laser cutting, and 18 eyes underwent filter bleb massage. Sixteen eyes who had filter bleb scarring received subconjunctival injection of 5-FU combined with bleb separation. One eye, 2 eyes and 2 eyes had iris neovascularization recurrence and increased IOP 2 months, 3 months and 5 months postoperatively, respectively, and the IOP decreased in 2 eyes after undergoing PRP. The other 3 eyes had uncontrolled IOP, and TCP was performed. One eye with uncontrolled IOP received a large dose of antiglaucoma medication and no light perception, and TCP was performed, which reduced IOP to normal at 2 months. One eye with high IOP that received ineffective antiglaucoma drugs received trabeculectomy at the 11th month.

The concentrations of VEGF-A and TGF-β1 in aqueous humour were 168.8±13.4 and 159.6±15.4 pg/ml after intravitreal injection, which were lower than the 160.2±7.6 and 151.9±2.3 pg/ml at baseline, respectively, and the differences were statistically significant (F=5.043, P=0.03; F=4.888, P=0.03). Although the concentration of PLGF in the aqueous humour of patients decreased from 30.9±1.0 pg/ml (baseline) to 30.5±1.1 pg/ml (post-injection), the difference was not statistically significant (F=1.376, P=0.25). The concentrations of VEGF-A, TGF-β1, and PLGF in the aqueous humour of NVG patients were positively correlated with iris neovascularization at baseline (r=0.919, P=0.000; r=0.925, P=0.000; and r=0.923, P=0.000, respectively). See Table 2.

Under light microscopy, trabecular meshwork was observed in 14 eyes of the 26 eyes that received intravitreal injection. We also note that the trabecular band became thinner with irregular shape; the endothelial cells of surface of trabecular band disappeared; the gap between trabecular meshworks narrowed or the trabecular band linked together; small amounts of melanin granules were seen among the trabecular bands; a small amount of blood cells surrounded adhered surgical specimens, which was unrelated to treatment; and trabecular tissue was not found in 12 eyes, which was associated with tissue not being cut intraoperatively or being embedded in the wrong direction (if the tissue was cut too far forward, the risk of bleeding, inflammation and ciliary body detachment increased) (Fig. 1a arrow).

Under light microscopy, the specimens without intravitreal conbercept showed different thicknesses of fibrous vascular membranes on the surface of the iris and many tiny neovascularizations on the iris stroma (Fig. 1b arrow). Neovascularization of the iris surface significantly regressed, and vessel blur remained (Fig. 1c, arrow). Small, thin-walled vessels in the anterior boundary layer and anterior stroma (Fig. 1d arrow) were displayed by specimens with intravitreal injection. Iris stroma atrophy and pigment cells that were disordered on the stroma were increased, which suggested synechia, which was related to high IOP and long-term application of hypotensive drugs [8].

Under transmission electron microscopy, iris tissue was observed in 26 eyes that received intravitreal injection, and these tissues showed that there was no obvious neovascularization on the surface of the iris (fig. 2a, arrow). Some neovascularization resisted in the iris stroma, and some vascular lumen became narrowed (fig. 2b, arrow) or atresic. The basement membrane of the blood vessel was not complete, and the material, such as red blood cells, in the lumen disappeared. Vascular endothelial cells degenerated (fig. 2b, arrow).

Discussion

NVG is a serious and refractory glaucoma with poor prognosis, and the pathogenesis of NVG has not been fully clarified [9-11]. Research has shown that the occurrence and development of angiogenesis or NVG are closely related to various kinds of cytokines such as VEGF, TGF-β1, interleukin-6, fibroblast growth factor, platelet-derived growth factor and others [12-15]. The members of the VEGF family are VEGF-A, VEGF-B, VEGF-C, VEGF-D and PLGF, and VEGF-A and PLGF have been shown to be associated with angiogenesis [12].

VEGF is released by retinal pigment epithelial cells in retinal ischaemia, and patients with NVG and its primary diseases have significantly higher concentrations of VEGF in their aqueous humour and vitreous [16]. VEGF combines with its -1 and -2 receptors, which produce vascular epithelium cell proliferation and migration and enhance vascular permeability, followed by vascular dilation and vascular reconstruction. As VEGF is upregulated, it can spread through the aqueous humour to the anterior segment of the eye, resulting in iris and anterior chamber angle neovascularization. Then, fibrovascular membranes are created, which is followed by synechia of the peripheral iris and trabecular meshwork that can inhibit the aqueous flow and lead to an increase in IOP [17-19].

Filtering surgery is the most effective method for the treatment of glaucoma [20], but if surgery is immediately performed for NVG patients, it is associated with unfavourable outcomes, such as hyphema, filter bubble adhesion and choroidal detachment due to neovascularizations on the iris and anterior chamber angle [21,22]. Horsely et al showed that intravitreal bevacizumab before trabeculectomy could regress iris neovascularization and reduce intraoperative and postoperative hyphaema [23].

Anti-VEGF agents can inhibit endothelial cell proliferation and angiogenesis through competitively inhibiting VEGF binding with its receptors [24]. Common anti-VEGF agents are pegaptanib, bevacizumab and ranibizumab. Pegaptanib can specifically combine with VEGF-165, and bevacizumab and ranibizumab can be combined with all subtypes of VEGF-A [25]. Another new agent is conbercept, which is a fusion protein derived from the extra-cellular domains of VEGF receptors -1 and -2 and the Fc portion of immunoglobulin G1. Conbercept shows high affinity with all subtypes of VEGF-A and VEGF-B and PLGF [26].

Surface iris neovascularization is derived from anterior chamber angle and stroma, but Yosuke et al showed that the effect of bevacizumab on the surface of the iris and stroma was different [27]. In our study, histopathological findings suggested that neovascularization regressed and damaged the structures of neovascularizations and reduced vascular permeability. Wakabayashi et al showed that the time for complete neovascularization regression is 1 day to 1 weeks after intravitreous bevacizumab [28], but long-term high IOP will produce irreversible damage to the optic nerve. Therefore, filtration surgery should be performed as soon as possible to reduce IOP and save visual function. The remaining atresia or cavity neovascularizations of the iris stroma will be reconstructed if the retinal ischaemia is not improved because of the timeliness of anti-VEGF agents (8 to 10 weeks), making PRP necessary [29].

We should regress neovascularization with intravitreal anti-VEGF agents first, and then trabeculectomy combined with PRP can be performed to reduce IOP and improve retinal ischaemia [30]. Studies have shown that trabeculectomy and PRP followed by intravitreal injection of bevacizumab or ranibizumab is safe and effective for NVG and produces fewer postoperative complications [31-33].

Oshima et al studied intravitreal bevacizumab, and after 2 months of follow-up, 29% cases had recurrent iris neovascularization [34]. Gheith et al found that 3 months and 5 months after intravitreal bevacizumab, iris neovascularization recurred [35]. Lin Zhaobin et al showed that 6 patients (27.3%) had recurrent iris neovascularization 4 months after intravitreal bevacizumab [36]. The recurrence of iris neovascularization maybe related to the timeliness of PRP and anti-VEGF agents, or a part of PRP energy were absorbed by muddy lens or the vitreous. In our study, the patients with recurrent iris neovascularization received PRP, anti-glaucoma drugs and TCP, and neovascularization regressed partially and IOP decreased.

In our study, IOP at the last visit was significantly lower than baseline IOP ($P < 0.05$), which is consistent with the results of Silva et al and Lin Zhaobin et al [29,36]. Satoko et al showed that high IOP and poor angle function at baseline in NVG patients were associated with uncontrolled IOP postoperatively [37]. In our study, baseline IOP in 19 eyes was higher than 60 mmHg, producing exudated depigmentation, which

caused serious postoperative pigment membrane reaction such that trabecular meshwork and filtration path were obstructed, and the angles of these patients were completely closed. Therefore, IOP was difficult to control. Four eyes received TCP, and the other 14 eyes were treated with anti-glaucoma medicine, filter bleb massage and other treatments, and 1 eye received trabeculectomy to reduce IOP.

In our study, after intravitreal anti-VEGF agents, the concentration of VEGF-A in aqueous humour decreased significantly, but the neovascularization of the iris and chamber angle were regressed incompletely. Therefore, we speculated that other cytokines may be involved in angiogenesis. PLGF has been shown to enhance angiogenesis by increasing vascular permeability, promoting inflammatory response and increasing the activity of VEGF-A. Therefore, PLGF may have a synergistic effect with VEGF-A in the development of NVG [12]. TGF-β1 plays an important role in cell growth, differentiation, apoptosis and immune regulation, embryonic development and wound repair and other biological activities. Previous studies confirmed that the concentrations of TGF-β1 in NVG patients were increased significantly, and TGF-β1 influenced trabecular meshwork, which resulted in decreased aqueous humour outflow and increased IOP, suggesting that TGF-β1 could play a role in the development of NVG [38,39] NVG may be the result of angiogenesis, immune response and inflammatory reactions. Anti-PLGF and anti-TGF-β1 treatment may be effective NVG treatments.

Conclusions

Intravitreal injection of conbercept combined with trabeculectomy and PRP can regress iris neovascularization, reduce intraoperative and postoperative complications, and effectively control IOP and preserve visual function. In our study, fewer cases and short follow-up time were our limitations. Therefore, we need a long-term, large sample study to observe the efficacy and safety of conbercept for the treatment of NVG and to provide new therapeutic targets for NVG.

Abbreviations

NVG: Neovascular glaucoma; BCVA: Best-corrected visual acuity; IOP: Intraocular pressure; ELISA: Enzyme-linked immunoabsorbent assay; CRVO: Central retinal vein occlusion; DR: Diabetic retinopathy; PRP: Panretinal photocoagulation; VEGF: Vascular endothelial growth factor; TGF-β1: Transforming growth factor-β1; PLGF: Placenta growth factor; LogMAR: Logarithm of the mimimum angle of resolution; MMC: MitomycinC; 5-FU: 5-Fluorouracil

Declarations

Ethics approval and consent to participate

The study was approved by the ethics committee of Tianjin Eye Hospital (No. TJYYLL-2015-15) and all procedures were performed in accordance with the Declaration of Helsinki, and all patients provided

voluntary informed consent to participate in the study. The study had obtained written consent from the participants.

Consent for publication

Not applicable.

Availability of data and materials

All data analysed during this study are included in this manuscript.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

LS: data collection, analysis and interpretation of data, drafting of the manuscript. JY:design, case selection, data interpretation, critical revision of the manuscript. JL: histopathological analysis of trabeculectomy specimens. All authors read and approved the final manuscript.

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References

1. Shazly TA, Latina MA. Neovascular glaucoma: etiolory, diagnosis and prognosis. Semin Ophthalmol. 2009;24:113-121.
2. Marra KV, Wagley S, Omar A, et al. Case-matched comparison of vitrectomy, peripheral retinal endolaser, and endocyclophotocoagulation versus standard care in neovascular glaucoma. Retina. 2015;35(6):1072-1083.

3. Nakatake S, Yoshida S, Nakao S, et al. Hyphema is a risk factor for failure of trabeculectomy in neovascular glaucoma: a retrospective analysis. BMC Ophthalmol. 2014;14:55.
4. Alkawas AA, Shahien EA, Hussein AM. Management of neovascular glaucoma with panretinal photocoagulation, intravitreal bevacizumab, and subsequent trabeculectomy with mitomycin C. J Glaucoma. 2010;19(9):622-626.
5. Jun YH, Tae HL, Mi SS, et al. Eifficacy and safety of intracameral bevacizumab for treatment of neovascular glaucoma. Korean J Ophthalmol. 2017;31(6):538-547.
6. Wilson MR, Mendis U, Smith SD, et al. Ahmed glaucoma valve implant vs trabeculectomy in the surgical treatment of glaucoma: a randomized clinical trial. Am J Ophthalmol. 2000;130(3):267-273.
7. Gedde SJ, Schiffman JC, Feuer WJ, et al. Treatment outcomes in the Tube Versus Trabeculectomy (TVT) study after five years of follow-up. Am J Ophthalmol. 2012;153(3):789-803.
8. Hamanaka T, Kasahara K, Takemura T. Histopathology of the trabecular meshwork and Schlemm's canal in primary angle-closure glaucoma. Invest Ophthalmol Vis Sci. 2011;52(12):8849-8861.
9. Distler JH, Hirth A, Kurowska-Stolarska M, et al. Angiogenic and angiostatic factors in the molecular control of angiogenesis. Q J Nucl Med. 2003;47(3):149-161.
10. Hong YR, Kim YH, Kin SY, et al. Plasma concentrations of vascular endothelial growth in retinopathy of prematurity after intervitreal bevacizumab injection. Retina. 2015;35(9):1772-1777.
11. Ferrara N, Henzel WJ. Pituitary follicular cells secrete a novel heparin-binding growth factor specific for vascular endothelial cells. Biochem Biophys Res Commun. 2012;425(3):540-547.
12. Chen SD, Zhou MW, Wang W ,et al. Levels of angiogenesis-related vascular endothelial growth factor family in neovascular glaucoma eyes. Acta Ophthalmol. 2015;93(7):e556-560.
13. Yu XB, Sun XH , Dahan E, et al. Increased levels of transforming growth factor-betal and –beta2 in the aqueous humor of patients with neovascular glaucoma. Ophthalmic Surg Lasers Imaging. 2007;38(1):6-14.
14. Bo QY, Zhang Y ,Wu YW, et al. Research advances of platelet derived growth factor family and its significance in neovascular eye diseases. Chin J Ophthalmnol. 2014;50(6):471-475.
15. Hou YH, Cui HP. Levels of aqueous and serum VEGF,TGF-β_1,IL-6 in neovascular glaucoma eye. Chin J Exp Ophthalmal. 2016;34(7):624-629.
16. Sivak-Callcott JA, Gass JD, et al. Evidence-based recommendations for the diagnosis and treatment of neovascular glaucoma. Ophthalmology. 2001;108(10):1767-1776.
17. Kwong TQ, Mohamed M. Anti-vascular endothelial growth factor therapies in ophthalmology: current use, controversies and the future. Br J Clin Pharmacol. 2014;78(4):699-706.
18. Chalam KV, Brar VS, Murthy RK. Human ciliary epithelium as a source of synthesis and secretion of vascular endothelial growth factor in neovascular glaucoma. JAMA Ophthalmol. 2014;132(11):1350-1354.
19. Noma H, Mimura T, Yasuda K, et al. Vascular endothelial growth factor and its soluble receptors-1 and -2 in iris neovascularization and neovascular glaucoma. Ophthalmologica. 2014;232(2):102-

109..

20. Rulli E, Biagioli E, Riva I, et al. Efficacy and safety of trabeculectomy vs nonpenetrating surgical procedures: a systematic review and meta-analysis. JAMA Ophthalmol. 2013;131(12):1573-1582.
21. Kiddee W, Tantisarasart T, Wangsupadilok B. Neovascular glaucoma: a retrospective review of 5-year experience in Songklanagarind Hospital. J Med Assoc Thai. 2012;95Suppl 4:S36-S42.
22. Hayreh SS. Neovascular glaucoma. Prog Retin Eye Res. 2007;26(5):470-485.
23. Horsley MB, Kahook MY. Anti-VEGF therapy for glaucoma. Curr Opin Ophthalmol. 2010;21(2):112-117.
24. Chen X, Li J, Li M, et al. KH902 suppresses high glucose-induced migration and sprouting of human retinal endothelial cells by blocking VEGF and PIGF. Diabetes Obes Metab. 2013;15(3):224-233.
25. Li X, Xu G, Wang Y, et al. Safety and efficacy of conbercept in neovascular age-related macular degeneration: results from a 12-month randomized phase 2 study: AURORA study. Ophthalmology. 2014;121(9):1740-1747.
26. Su L, Ren X, Wei H, et al. Intravitreal conbercept (KH902) for surgical treatment of severe proliferative diabetic retinopathy. Retina. 2016;36(5):938-943.
27. Sugimoto Y, Mochizuki H, Okumichi H, et al. Effect of intravitreal bevacizumab on iris vessels in neovascular glaucoma patients. Graefes Arch Clin Exp Ophthalmol. 2010;248(11):1601-1609.
28. Wakabayashi T, Oshima Y, Sakaguchi H, et al. Intravitreal bevacizumab to treat iris neovascularization and neovascular glaucoma secondary to ischemic retinal diseases in 41 consecutive cases. Ophthalmology. 2008;115(9):1571-1580.
29. Silva P, Jorge R, Alves C, et al. Short-term results of intravitreal bevacizumab (Avastin) on anterior segment neovascularization in neovascular glaucoma. Acta Ophthalmol Scand. 2006;84(4):556-557.
30. Huang P, Wang WQ, Shi Y, et al.Comparison of efficacy between intravitreal bevacizumab combined with trabeculectomy and intravitreal bevacizumab combined with cyclophotocoagulation for late stages neovascular glaucoma.Chin J Exp Ophthalmol. 2015,33(4):362-366.
31. Kobayashi S, Inoue M, Yamane S, et al. Long-term Outcomes After Preoperative Intravitreal Injection of Bevacizumab Before Trabeculectomy for Neovascular Glaucoma. J Glaucoma. 2016;25(3):281-284.
32. Elmekawey H, Khafagy A. Intracameral ranibizumab and subsequent mitomycin C augmented trabeculectomy in neovascular glaucoma. J Glaucoma. 2014;23(7):437-440.
33. Kitnarong N, Sriyakul C, Chinwattanakul S. A Prospective Study to Evaluate Intravitreous Ranibizumab as Adjunctive Treatment for Trabeculectomy in Neovascular Glaucoma. Ophthalmol Ther. 2015;4(1):33-41.
34. Oshima Y, Sakaguchi H, Gomi F, et al. Regression of iris neovascularization after intravitreal injection of bevacizumab in patients with proliferative diabetic retinopathy. Am J Ophthalmol. 2006;142(1):155-158.

35. Gheith ME, Siam GA, de Barros DS, et al. Role of intravitreal bevacizumab in neovascular glaucoma. J Ocul Pharmacol Ther. 2007;23(5):487-491.
36. Lin ZB, Wu XM, Zhu MH, et al. The efficacy of intravitreal injection of bevacizumab combined with trabculectomy for the management of neovascular glaucoma. Chin J Pract Ophthalmol. 2013;31(10):1291-1294.
37. Nakano S, Nakamuro T, Yokoyama K, et al. Prognostic Factor Analysis of Intraocular Pressure with Neovascular Glaucoma. J Ophthalmol. 2016;2016:1205895.
38. Guo B, Fan QH, Chen Q, et al. Levels of transforming growth factor-β_2 in nevascular glaucoma eyes and their associated diathesis. J Clin Ophthalmol. 2011;19:122-126.
39. Prendes MA, Harris A, Wirostko BM , et al. The role of transforming growth factor beta in glaucoma and the therapeutic implications. Br J Ophthalmol. 2013;97(6):680-686.

Tables

Table 1 IOP, BCVA and the number of antiglaucoma medications for NVG patients

time	eyes	IOP (mmHg)	BCVA(LogMAR)	antiglaucoma medications
baseline	52	48.1±14.2	2.3±1.1	3.0(3.0, 4.0)
after injection	52	44.3±14.0	2.2±1.1	3.0(3.0, 4.0)
1 day	52	19.5±10.6[a]	2.2±1.1	0.0(0.0, 0.0)[a]
1 week	49	16.1±9.3[a]	1.9±1.0	0.0(0.0, 0.0)[a]
1 month	42	20.2±8.9[a]	2.0±1.1	0.0(0.0, 0.0)[a]
3 months	40	23.1±10.6[a]	2.0±1.2	0.0(0.0, 0.8)[a]
6 months	39	25.1±8.6[a]	2.0±1.2	0.0(0.0, 1.0)[a]
1 year	39	23.2±8.7[a]	2.1±1.2	1.0(0.0, 1.0)[a]
F/χ^2		878.486	163.157	294.232
P		0.000	0.000	0.000

Compared with pre-injection，[a] $P<0.05$，ANOVA，Dunnett-t test，Kruskal-Wallis test

Table 2 The concentration of VEGF-A, PLGF, and TGF-β1 (pg/ml)

	time		*F*	*P*
	pre-injection	post-injection		
VEGF-A	168.8±13.4	160.2±7.6 [a]	5.043	0.03
PLGF	30.9±1.0	30.5±1.1	1.376	0.25
TGF-β1	159.6±15.4	151.9±2.3 [a]	4.888	0.03

Compared with pre-injection [a] $P<0.05$. ANOVA LSD -*t* test

Figures

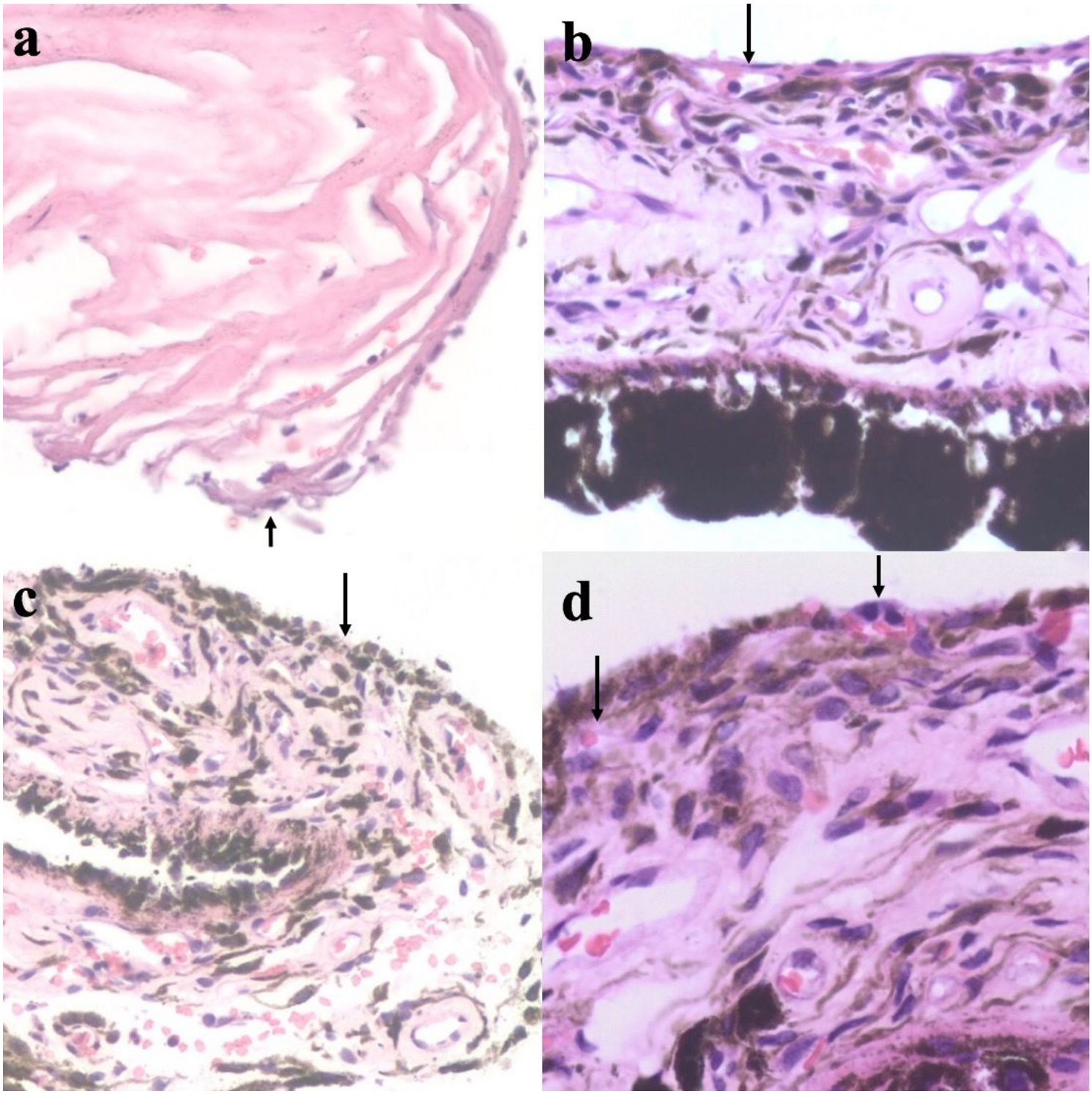

Figure 1

Light microscopy of the trabecular meshwork (a) and iris (c,d) with intravitreal conbercept, iris (b) without intravitreal conbercept, a,b,c,d: HE × 200.

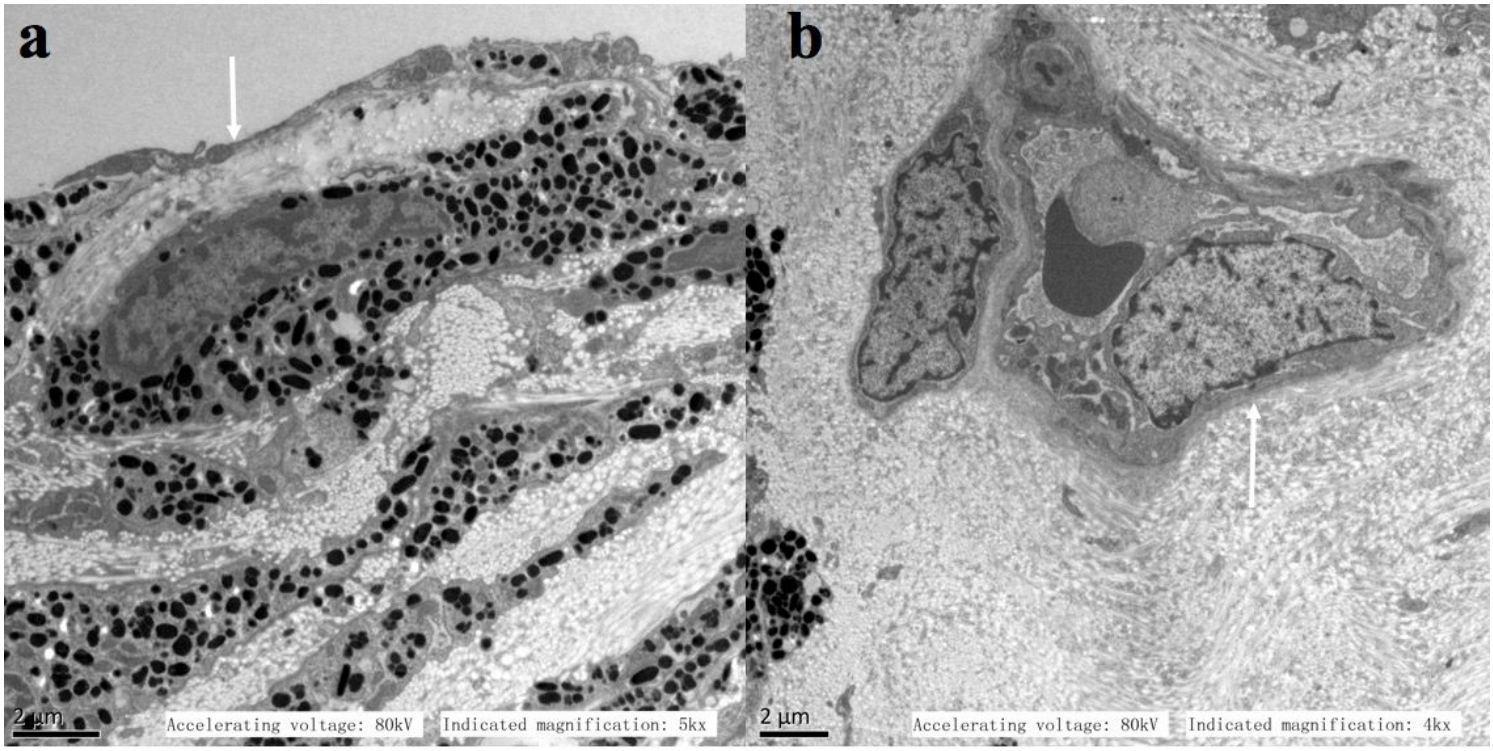

Figure 2

Transmission electron microscopy of the iris with intravitreal injection. a: ×5000, bar=2 μm; b: ×4000, bar=2 μm.