Effect of Intracameral Bevacizumab Injection on Corneal Endothelial Cells: An In Vivo Evaluation

Mohamed H. Hosny, Mohamed A. Zayed, Ahmad M.M. Shalaby, and Iman M. Eissa

Abstract

Background: We studied the effect of an intracameral bevacizumab injection on the corneal endothelium and iris neovessels (INV).

Methods: Ten eyes were studied in 10 patients with an average age of 52.1 ± 13.17 years. Patients underwent intracameral bevacizumab injections at a concentration of 1.25 mg/0.05 mL. Intraocular pressure, best-corrected visual acuity, gonioscopy, neovascular membrane extent, anterior segment photography, iris fluorescein angiography, pachymetry, and specular microscopy were recorded preoperatively and postoperatively.

Results: The minimum follow-up period was 4 months. INVs started to regress within the first 2 days after the injection and regressed completely by the end of the fourth week. Reduction in INV leakage started 1 week after injection, and resolved in 8 eyes (80%) by the end of the fourth week. Intraocular pressure dropped significantly from 17.8 ± 4.8 mmHg to 16.6 ± 2.8 mmHg over 4 weeks. The mean endothelial cell loss was 3.95% ± 6.78%.

Conclusions: An intracameral bevacizumab injection proved to be safe for corneal endothelial cells.

Introduction

Vascular endothelial growth factor (VEGF) has been identified as an endothelial cell-specific mitogen and an inducer of angiogenesis in vivo. VEGF has been implicated as the major angiogenic stimulus for intraocular neovascularization in certain ocular diseases, including proliferative diabetic retinopathy, age-related macular degeneration, retinal vascular occlusions, choroidal neovascularization, and other ischemic retinal diseases.1–5

Antivascular endothelial growth factor (anti-VEGF) treatment modalities aimed at stopping choroidal angiogenesis in order to improve vascular permeability have revolutionized clinical practice for the above-mentioned neovascular eye diseases.6 New intravitreous and intracameral applications of anti-VEGF have recently been described in the treatment of neovascularization of the anterior segment.7, 8 In preliminary studies, the anti-VEGF agents, pegaptanib sodium, ranibizumab, bevacizumab, VEGF trap, and bevasiranib have shown promise in the treatment of these diseases.9–11

Bevacizumab is a full-length, humanized, anti-VEGF monoclonal antibody that binds to all forms of VEGF-A. It is the first antiangiogenic agent approved by the Food and Drug Administration for the treatment of metastatic colorectal cancer.12, 13

Current studies are actively investigating the efficacy and safety of anti-VEGF agents, either alone or combined with standard treatments (eg, laser photocoagulation), anti-inflammatory agents, or other non-VEGF-based antiangiogenic therapies.6

In this study, a series of patients with anterior segment neovascularization were treated with intracameral bevacizumab (ICB), either alone or as an adjunct to other modalities to control the disease.

Methods

The study was performed on 10 eyes of 10 patients that presented to the outpatient clinic of the ophthalmic diagnostic and laser unit of Cairo University Hospitals. The mean age of the patients was 52.1 ± 13.17 years (range 17–62). Seven patients were males (70%) and 3 were females (30%). Anterior segment neovascularization was caused by proliferative diabetic retinopathy in 7 patients, central retinal vein occlusion in 2 patients, and alkali chemical in inquiry 1 patient.

All patients underwent an intracameral bevacizumab (Avastin; Genetech Inc, South San Francisco, CA) injection of 1.25 mg/0.05 mL through a temporal paracentesis under topical anesthesia in the operating room. All patients were examined and graded by the same observer. Intraocular pressure (Goldmann applanation tonometry), best-corrected visual acuity, gonioscopy, neovascular membrane (NVM) extent, anterior segment photography, iris fluorescein
angiography, pachymetry, and specular microscopy were recorded on the patient’s chart preoperatively and postoperatively. The Noncon Robo Pachy Model SP-9000LC specular microscope (Konan Medical Corporation, Miyanishi-cho, Nishinomiya, Hyogo, Japan) was used to record the central corneal endothelial image and measures the corneal thickness. A KSS-300 Image Storage System (Konan Medical Corporation, Miyanishi-cho, Nishinomiya, Hyogo, Japan) was used to capture and save the image from the Noncon Robo specular microscope. The KSS-300 Image Storage System was later used to analyze the corneal endothelial image, using the center method after counting a minimum of 10 cells in each eye. INV and leakage from the iris neovessels were graded clinically as previously described in the literature and in Tables 1 and 2.\textsuperscript{12,13}

**Results**

The minimum follow-up period for all patients was 4 months. All patients tolerated the injection well with no significant adverse ocular events (corneal toxicity, uveitis, endophthalmitis) or systemic events (elevated blood pressure).

Six patients had received prior panretinal photocoagulation for active retinal neovessels or INVs associated with diabetic retinopathy\textsuperscript{5} or CRVO.\textsuperscript{1} The other 4 patients received ICB injection as a primary therapy. The INVs present preoperatively started to regress clinically within the first 2 days postoperatively, then decreased in caliber gradually, until they regressed completely by the end of the fourth week. All patients showed partial reductions in INV leakage 1 week after the injection. Complete resolution of INV leakage was noted in 8 eyes (80\%) by the end of the fourth week (Fig. 1). Two patients showed only partial regression at 4 weeks (Fig. 2), and it was resolved completely by the end of the sixth week. Another 2 patients had a recurrence of leakage from INVs 6 weeks after the ICB injection; these patients were given another ICB injection that controlled the INVs regarding both extent and leakage till the end of follow-up.

Improvement in visual acuity was minimal. The mean preoperative best-corrected visual acuity (BCVA) was 0.17 ± 0.11 (range, hand motion to 0.3). The mean postoperative BCVA was 0.2 ± 0.10 (range 1/60–0.3). This minimal difference was not statistically significant ($P = 0.05$, paired sample $t$-test). Intraocular pressure dropped from 17.8 ± 4.8 mmHg (range 12–28) preoperatively to 16.6 ± 2.8 mmHg (range 13–20) ($P = 0.19$, paired sample $t$-test) postoperatively. Intraocular pressure was stable throughout the follow-up period, with a topical beta blocker and carbonic anhydrase inhibitor eye drops given twice daily.

The mean endothelial cell loss at the end of the fourth month follow-up was 3.95% ± 6.78% (range 1.54–23.22). The mean preoperative endothelial cell density was 2,631.5 ± 412.57/μm\textsuperscript{2} (range 1,718–3,048). Postoperatively, it dropped to 2,547.6 cells ± 503.86/μm\textsuperscript{2} (range 1,319–2,993). This was statistically significant ($P = 0.04$, paired sample $t$-test). The maximum decrease was observed in one patient that had INVs complicated with ocular trauma.

Regarding the endothelial cell morphology, the percentage of hexagonal cells (pleomorphism) decreased from 66.2% ± 8.22% (range 48–79) to 58.2% ± 9.34% (range 40–74). This was statistically significant ($P = 0.03$, paired sample $t$-test). The coefficient of variation (CoV) (polymegathism), changed from 27.6 ± 9.91 (range 21–55) to 31.2 ± 6.96 (range 22–45), a change that was not significant statistically ($P = 0.25$, paired sample $t$-test). The standard deviation of cell size increased from 108.2 ± 75.35 (range 77–322) to 120.6 ± 122.97 (range 73–470) with the maximum change again noted in the patient in whom trauma was the underlying cause of neovascularization. This change was still not statistically significant ($P = 0.43$, paired sample $t$-test). After injection, 9 patients (90\%) had a standard deviation of cell size of <140, the maximum recommended by the KSS-300 manual.

Clinically the corneal transparency was not affected, with no detectable corneal edema. This was confirmed by the pachymetry values obtained from the analysis of the Noncon Robo Pachy Model SP-9000LC specular microscope images. The mean preoperative pachymetry was 557.3 microns ± 26.8 (range 531–617). The mean postoperative pachymetry was 555.1 μ ± 25.08 (range 523–599). This was not statistically significant ($P = 0.59$, paired sample $t$-test).

**Discussion**

The management of neovascular eye diseases has been a challenge for ophthalmologists. Visual outcome has been poor in a significant proportion of cases. Retinal ischemia results in hypoxia. Hypoxia, in turn, stimulates the release

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**Table 1. Grading of Iris Neovascularization**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Iris neovascularization</th>
</tr>
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<tbody>
<tr>
<td>Grade 0</td>
<td>No iris neovascularization</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Fine surface neovascularization of the pupillary zone of the iris involving less than 2 quadrants</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Surface neovascularization of the pupillary zone of the iris involving more than 2 quadrants</td>
</tr>
<tr>
<td>Grade 3</td>
<td>In addition to neovascularization of the pupillary zone, neovascularization of the ciliary zone of the iris and/or ectropion uvea involving 1 to 3 quadrants</td>
</tr>
<tr>
<td>Grade 4</td>
<td>In addition to neovascularization of the pupillary zone, neovascularization of the ciliary zone of the iris and/or ectropion uvea involving more than 3 quadrants</td>
</tr>
</tbody>
</table>

**Table 2. Grading of Iris Fluorescein Angiography**

<table>
<thead>
<tr>
<th>Grade</th>
<th>Iris fluorescein angiography findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grade 0</td>
<td>No fluorescein leakage</td>
</tr>
<tr>
<td>Grade 1</td>
<td>Mild fluorescein leakage in 1 or 2 quadrants of the pupillary sphincter</td>
</tr>
<tr>
<td>Grade 2</td>
<td>Mild fluorescein leakage in 3 or 4 quadrants of the pupillary sphincter</td>
</tr>
<tr>
<td>Grade 3</td>
<td>Pupillary-sphincter leakage in 3 or 4 quadrants combined with leakage in 1 or 2 quadrants of the iris stroma</td>
</tr>
<tr>
<td>Grade 4</td>
<td>Pupillary-sphincter leakage in 3 or 4 quadrants combined with leakage in at least 3 quadrants of the iris stroma</td>
</tr>
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of angiogenic factors that lead to ocular neovascularization. Thus, all previous treatment modalities have been directed toward combating ischemia, and for this, panretinal laser photocoagulation is the treatment of choice.

Vascular endothelial growth factor (VEGF) is the main regulator of pathological ocular neovascularization. Significantly raised levels of VEGF have been demonstrated in patients with rubeosis, neovascular glaucoma, and other ocular neovascular diseases.

The emergence of anti-VEGF has given rise to new treatment modalities for neovascularization. Of note, there is a time lag for the development of neovascularization after ablation of the hypoxic retina. This allows for progression of iris rubeosis and growth of the NV, which result in more angle closure and an increase in the severity of neovascular glaucoma. An intravitreous anti-VEGF injection has been used previously to treat posterior segment neovascularization, and more recently, to treat neovascularization of the anterior segment. Most recently, an intracameral injection of anti-VEGF was used to control neovascularization in the anterior segment.

The ICB injection has been proven safe in a rabbit experimental model. To our knowledge, its effect on the cornea has not been studied previously. In this study, in addition to routine ophthalmological assessments pre- and post ICB injection, we studied corneal endothelial cell loss by performing specular microscopy (Fig. 3).

We found that the BCVA showed a minimal, but significant improvement with the ICB injection. The minimal improvement in BCVA could be attributed to the age of the patient population. All the patients had either cataracts or an underlying retinal pathology that affected visual acuity. The youngest patient (17 years old) had a traumatic cataract. The change in visual acuity was comparable to that described in previous reports.

Before treatment, the severity of neovascular glaucoma was minimal. None of our patients showed an IOP that
exceeded 28 mmHg (average 17.8 ± 4.8 mmHg) with the combination of beta blocker and carbonic anhydrase inhibitors. All patients had an open angle, grade 1 or 2. The IOP also showed a minimal, but significant improvement after the ICB injection with the same combination treatment. This could be attributed to the regression of neovascularization. Due to the differences in patient characteristics, we cannot compare our results to those published in previous studies.26,27 In those studies, the IOP reached 51 mmHg, and some patients were candidates for glaucoma surgery.

We studied corneal toxicity with specular microscopy. We found that the mean endothelial cell count dropped after the ICB injection. The cell loss was statistically significant, but had no clinical effect on the cornea in the form of corneal edema. We also relied on the pachymetry values that showed a non-statistically significant change before ICB injection or 4 months after ICB injection. Before injection the mean pachymetry was 557.3 μ ± 26.8 (range 531–617), while post-injection it was 555.1 μ ± 25.08 (range 523–599).

To our knowledge, no previous studies on ICB injections evaluated endothelial cell loss. The endothelial cell loss observed in our study was minimal compared to the endothelial cell loss after cataract surgery (range 8%–11.2%),24,25 or to that after glaucoma surgery (range 0.2%–14.9%), depending on the technique.26,27 The maximal loss was noted in one patient with an underlying pathology of trauma; this alone might have affected the integrity of the endothelium. This particular patient also had the lowest pre-injection endothelial cell count (1,718 cells/mm²). Kim and coauthors27 reported high endothelial cell loss after Ahmed glaucoma valve implantation. That study also reported a lower pre-operative endothelial cell count (<1,700 cells/mm²). In our study, one or both factors could have explained the high endothelial cell loss in the patient with maximal cell loss. We did not exclude this patient from the study because we wanted to evaluate the response to ICB after trauma. Even with high endothelial cell loss, the cornea remained clinically clear in this patient. Excluding this patient from the statistical analysis reduced the average cell loss to 1.8%.

We also studied the endothelial cell morphology. The only significant change was in the percentage of hexagonal cells (pleomorphism). Before ICB injection, it was 66.2% ± 8.22% (range 48–79). This dropped to 58.2% ± 9.34% (range 40–74) after ICB (P = 0.03, paired sample t-test). The changes in the coefficient of variation (CoV) and standard deviation of cell size were still not statistically significant (P = 0.25 and 0.43, respectively, paired sample t-test). According to the KSS-300 manual, the standard deviation of cell size should be <1.40. This was the case in 9 patients after ICB injection. Again, the maximum change in cell size standard deviation and coefficient of variation was noted in the patient in whom the neovascularization was caused by trauma. All the measurements were done by a single observer to eliminate interobserver variability. These changes suggest again that the procedure was safe on the corneal endothelial cells.

The INVs showed marked improvement both clinically and angiographically. All patients showed regression of INVs by the end of the fourth week after ICB injection. Leakage in iris fluorescein angiography resolved in 80% of the eyes by the end of the fourth week and in all eyes (100%) by the end of the sixth week. We had to re-inject 2 eyes (20%) after 6 weeks to control recurrent leakage. This was achieved till the end of the follow-up period. Both patients were longstanding diabetics with poor control of the disease, and were undergoing ICB injection after multiple sessions of panretinal photocoagulation. We owe the need of a repeated injection to the severity of the disease at presentation as well as its duration rather than whether the ICB injection was performed as a primary or a secondary procedure. In their study on 16 eyes, Chalam and coauthors21 had to re-inject 2 eyes 2 weeks after the initial procedure, and were left with one eye with unresolved leakage from INVs.

The findings from our study were comparable to those from previous studies. Moreover, we presented a study of the corneal endothelium by specular microscopy. To our knowledge, this has not been tested in vivo previously. Based on our results, we encourage the use of ICB injection in favor of the intravitreal injection. Compared to the intravitreal injection, the ICB injection provides a lower risk of bleeding, because the needle is introduced into the anterior chamber under direct visualization. The ICB can also be used as an adjunct to other modalities, including panretinal argon laser photoocoagulation, to control intraocular neovascularization.

Our study was limited by the small number of eyes included. We are currently conducting a study on the use of the ICB injection in a larger number of patients to confirm its safety on corneal endothelial cells.

References


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Address correspondence to:
Dr. Mohamed H. Hosny
Ophthalmology Clinic
Cairo University
84 Shehab Street
Mohandeseen
Giza
Egypt
E-mail: gozreem@yahoo.com
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