Concentration of Vascular Endothelial Growth Factor After Intracameral Bevacizumab Injection in Eyes With Neovascular Glaucoma

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**Purpose:** To study the concentration of vascular endothelial growth factor (VEGF) in the aqueous humor before and after intracameral injection of bevacizumab in eyes with neovascular glaucoma, and to detect the duration of an anti-VEGF effect of bevacizumab in the anterior chamber.

**Methods:** In this prospective interventional case series, 1.25 mg of bevacizumab was injected into the anterior chamber of five eyes in five neovascular glaucoma patients. Aqueous humor samples were obtained just before intracameral injection of bevacizumab and two weeks after injection. The concentrations of VEGF in the aqueous humor were measured using ELISA. To investigate corneal endothelial damage after intracameral bevacizumab injection, specular microscopy was performed before injection and two weeks after injection. Slit lamp photo and iris fluorescent angiography was performed to determine the regression of iris neovascularization.

**Results:** After injection, substantial regression of neovascularization or fluorescein leakage was seen in all treated eyes. The VEGF concentrations in the aqueous humor in eyes with NVG were 1181.8±1248.3 pg/mL before intracameral injection of bevacizumab. Two weeks after injection, the VEGF concentrations decreased to 33.2±12.2 pg/mL (p=0.04, Wilcoxon signed rank test). There were no significant changes in IOP or corneal endothelial cells.

**Conclusions:** Intracameral bevacizumab injection can remarkably reduce iris neovascularization in neovascular glaucoma patients. VEGF levels were significantly decreased two weeks after injection and corneal toxicity was not observed during short term follow-up.


**Key Words:** Bevacizumab, Intracameral injection, Neovascular glaucoma, Vascular endothelial growth factor

**Materials and Methods**

After obtaining informed consent, we collected operating...
Fig. 1. Vascular endothelial growth factor (VEGF) in the aqueous humor of eyes with neovascular glaucoma. VEGF levels are shown before intracameral bevacizumab injection (pre-bevacizumab), two weeks after injection (post-bevacizumab) and for the control group.

Table 1. Changes in cell density and morphology of corneal endothelial cells after intracameral bevacizumab injection

<table>
<thead>
<tr>
<th>Corneal endothelial cell density (number/mm²)</th>
<th>Coefficient of variation⁰ (%) (range)</th>
<th>Hexagonality¹ (%) (range)</th>
</tr>
</thead>
<tbody>
<tr>
<td>pre-bevacizumab</td>
<td>2246±552</td>
<td>37.8 (34-49)</td>
</tr>
<tr>
<td>post-bevacizumab</td>
<td>2254±589</td>
<td>38.2 (34-52)</td>
</tr>
</tbody>
</table>

⁰ Coefficient of variation = standard deviation of area of endothelial cell/average area of endothelial cell × 100; ¹ Hexagonality = percentage of hexagonal cells. No statistically significant difference by Wilcoxon signed rank test p > 0.05.

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room samples of aqueous humor from five human subjects (age range, 43-87 years; mean, 64.2+13.1). Clinically, three of the patients had a proliferative diabetic retinopathy and two had a CRVO. All patients suffered from neovascular glaucoma due to retinal ischemia. Complete sessions of laser photoagulation were performed on the ischemic retinas of all patients and bevacizumab was injected intravitreally on three of five eyes during the outpatient follow up period, but no therapeutic intervention on the retina was done within six months before and two weeks after first intracameral bevacizumab injection. On gonioscopic examination, three patients had peripheral anterior synchia of about 120 degrees and angles were partially opened. Another two patients had peripheral anterior synchia around 360 degrees with closed angle. The mean intraocular pressure (IOP) was 29.2±10 mmHg (16 to 44 mmHg). All of the patients had used two to three anti-glaucoma drugs to lower intraocular pressure and maintained use of these medications after intracameral bevacizumab injection.

Aqueous humor was also sampled before cataract surgery from eight eyes in eight patients (age range, 70-86 years mean, 67.4+10.8) with cataracts who did not have diabetes mellitus or other ocular diseases. After the eye had been prepared in a standard fashion using 5% povidone/iodine and topical antibiotics, we obtained 0.1 to 0.2 ml of undiluted aqueous humor by limbal paracentesis using a 30-gauge needle attached to a microsyringe. We aspirated the aqueous humor within two to five seconds from the central pupillary area without touching the iris, lens, or corneal endothelium. The samples were placed immediately in liquid nitrogen and stored at -70°C until analyzed. A total of 0.05 mL (1.25 mg) of undiluted bevacizumab was injected intracameral through the limbus. After the injection, IOP and retinal artery perfusion were assessed, and patients were instructed to administer topical antibiotics (levofloxacin) for three days.

Aqueous sampling and bevacizumab injection were repeated after two weeks. Patients with established NVG were treated with medical and/or surgical therapy as needed. Ophthalmic evaluation included complete ophthalmic examination including degree of iris neovascularization and IOP, iris angiography, specular microscopy and slit lamp photography. The patients’ records were reviewed to record patient demographics and clinical data.

Total VEGF concentrations were determined by ELISA (Cat. DVE00, R&D Systems, Minneapolis, MN USA) according to the manufacturer’s instructions. The intensity of color developed was measured using an ELISA reader (MQX-200, BioTEK Instruments, Inc.) at 450 nm optical density (OD) with correction at 570 nm.

Before injection and two weeks after injection, corneal endothelial density, coefficient of variation and hexagonality were measured with specular microscopy, and intraocular pressure was measured with a Goldmann applanation tonometer.

Results

The mean VEGF concentration in the aqueous humor in eyes with NVG was 1181.8±1248.3 pg/mL (46.0 to 2745.7 pg/mL) before intracameral injection of bevacizumab and that of the control group was 20.7±12.4 pg/mL. Two weeks after injection, the VEGF concentrations in the test group had decreased to 33.2±12.2 pg/mL (14.0 to 43.7 pg/mL) (p=0.04) (Fig. 1). IOP was 29.2±10.1 mmHg before bevacizumab injection, 30.8±8.4 mmHg right after injection and dropped to 24.0±7.3 mmHg 2 weeks after injection, but the differences in these levels were not significant.

On specular microscopy examination, corneal endothelial density, coefficient of variation and hexagonality were 2246±552, 37.8±6.3, 52.8±12.1 respectively before bevacizumab injection and 2254±589, 38.2±8.1, 49.2±12.1 respectively two weeks after injection (p>0.05) (Table 1). High-quality iris fluorescein angiographs were obtained before and after intracameral bevacizumab injection in five eyes.

Table 1. Changes in cell density and morphology of corneal endothelial cells after intracameral bevacizumab injection