Angiographic Changes in Iris and Iridocorneal Angle Neovascularization After Intravitreal Bevacizumab Injection

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Objective: To evaluate the effects of the intravitreal (IV) injection of bevacizumab on anterior segment neovascularization using anterior segment angiography.

Methods: We observed 1 eye with iris and iridocorneal angle neovascularization and 3 with neovascular glaucoma from 4 patients with diabetic retinopathy in 3 eyes and central retinal vein occlusion in 1 eye. Two healthy eyes from 2 other patients served as control eyes. Three eyes, including 1 normal eye, were examined by iris angiography; the other eyes underwent iridocorneal angle angiography with fluorescein (FA) and indocyanine green (IA) using a Heidelberg Retina Angiograph 2. After angiography, 4 eyes with neovascularization were treated with IV bevacizumab (1.25 mg per 0.05 mL) and underwent angiography once more 4 to 6 days after treatment.

Results: Iris angiography with indocyanine green revealed many iris vessels, but not dye leaking, in both normal and glaucomatous eyes, and the angiography with fluorescein showed intensive vessel leakage in the iris as well as iridocorneal angle neovascularization, but not in normal eyes. Angle angiography revealed vessel structures with indocyanine green and intensive leakage with fluorescein in the iris and showed iridocorneal angle neovascularization and neovascular glaucoma, whereas no vessel structures appeared with IA or FA in the normal eye. After IV bevacizumab injection in eyes with neovascularization, the vascular structure did not change with IA, but dye leakage remarkably decreased with FA in the iris and angle. However, newly formed vessels in the iris and iridocorneal angle seemed to disappear on slitlamp examination.

Conclusion: Intravitreal injection of bevacizumab effectively reduces vascular permeability, whereas newly formed vessels are still present in the iris and iridocorneal angle.

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Neoangiogenic Glaucoma (NVG) is a serious complication associated with retinal ischemic changes, such as diabetic retinopathy and central retinal vein occlusion. The etiology of intraocular pressure (IOP) elevation in patients with NVG and an open iridocorneal angle is thought to arise from aqueous outflow blocking, which may be caused by the development of a fibrovascular membrane covering the anterior surface of the trabecular meshwork or by invasion of the intertrabecular spaces by newly formed vessels (NFVs), although the details of these mechanisms are still unclear.

Vascular endothelial growth factor has been implicated as a key molecule for the development of NFVs and NVG. Bevacizumab (Avastin; Roche, Basel, Switzerland) is a full-length humanized anti-vascular endothelial growth factor monoclonal antibody that is approved for use as an antiangiogenic agent for metastatic colorectal cancer. Recent studies have reported that the off-label use of intravitreal (IV) injection of bevacizumab is an effective and safe treatment for NVG. Intravitreal injection of bevacizumab leads to a dramatic regression of the new iris and angle vessels and a decrease in IOP, although these effects may be transient owing to the drug's short half-life. The effects of bevacizumab on the vasculature of the anterior ocular segment, however, are unknown.

A previous study reported that fluorescein angiography (FA) and indocyanine green angiography (IA) in the
anterior ocular segment are useful for studying the structure and hemodynamics of NFVs, which is helpful in evaluating the effect of IV bevacizumab injection on iris and angle neovascularization.

This study was conducted to evaluate the effect of IV bevacizumab injection on anterior segment neovascularization in patients with NVG using anterior segment angiography (Heidelberg Retina Angiograph 2 [HRA 2]; Heidelberg Engineering, Heidelberg, Germany).

**METHODS**

This study conducted FA and IA simultaneously in the anterior segment of 6 eyes from 6 patients (Table 1) using an HRA 2. Two eyes (from patients 1 and 2) had no ocular disease, including intraocular neovascularization, and therefore served as control eyes. Another eye (from patient 3) had iris and iridocorneal angle neovascularization without NVG, and the other three (from patients 4-6) had NVG with an open iridocorneal angle. The IOP in patient 3 was within the reference range, whereas the others had an elevated IOP. No patient had a history of corneal disease, uveitis, severe heart disorders, or cerebral infarction. The investigation was conducted in accordance with the Declaration of Helsinki on biomedical research involving human subjects and was approved by the institutional review board of the University of Occupational and Environmental Health, Japan. After the nature of the study was explained to the participants, all provided their written informed consent.

**ANTERIOR SEGMENT ANGIOGRAPHY**

None of the participants had any drug allergies, and their blood pressures and pulse rates were all within reference ranges. Three eyes (in patients 1, 3, and 4) underwent iris angiography, and the other 3 (in patients 2, 5, and 6) received iridocorneal angle angiography with the HRA 2. All eyes with iris and angle neovascularization underwent a second angiography after IV bevacizumab injection. A Goldmann gonioscope was used for iridocorneal angle angiography. Injection of metoclopramide hydrochloride, 10 mg, through an intravenous catheter was followed by intravenous injection of a mixed solution of fluorescein (2.5 mL) and indocyanine green (2.5 mL) through the same route. The angiographic pictures were stored on a hard disc as digital images with the HRA 2.

**IV INJECTION OF BEVACIZUMAB**

Intravitreal injection of bevacizumab was performed on 4 patients with NVG on the day of or the day after angiography. Slitlamp and gonioscopic examinations and IOP measurements were performed before treatment. After topical anesthesia (0.4% oxybuprocaine hydrochloride), 1.25 mg/0.05 mL bevacizumab (100 mg/4 mL) was injected intravitreally into the superotemporal quadrant, 4 mm posterior to the limbus, under aseptic conditions. The patients were instructed to use a topical antibiotic (levofloxacin) 4 times daily for 3 days before and 1 week after the injection. Slitlamp examination and IOP measurement were followed by angiography 4 to 6 days after the treatment.

**RESULTS**

**EYES WITHOUT OCULAR NEOVASCULARIZATION**

Slitlamp microscopy revealed no vessels in the iris and iridocorneal angle in the control eyes (patients 1 and 2). Iris vessels appeared with FA and IA 20 seconds after dye injection. The iris vessels, however, were mostly blocked with melanin pigment in FA. Indocyanine green angiography showed clearer images and more vessels than did FA (Figure 1A and B). The iridocorneal angiography showed no vascular structures in the trabecular meshwork, although the iris root had vessel structures with both FA and IA (Figure 1C and D). There was no dye leakage from the iris vessels in either FA or IA.
EYES WITH NFVs IN THE ANTERIOR SEGMENT

Clinical Course

Slitlamp binocular microscopy with gonioscopy showed a clearly visible neovascularization of the iris and vessels crossing the ciliary body band within the iridocorneal angle (Figure 2A and C). The NFVs in the iris and iridocorneal angle were not observed during slitlamp examinations 4 to 6 days after the injection (Figure 2B and D).

Iris Angiography

Vessel images appeared at approximately 23 to 30 seconds after the injection with both FA and IA, first at the pupillary margin and then at the surface of the iris for 2 eyes undergoing iris angiography (patients 3 and 4). The FA and IA images were similar in the early stage of angiography, although the vascular structure was clearer with IA than with FA. Fluorescein angiography showed marked leakage from NFVs in patchy images,
whereas IA maintained linear images without dye leakage (Figure 3A and B).

After IV bevacizumab injection, FA showed that dye leakage from the NFVs decreased remarkably compared with the preinjection levels, whereas IA showed that there were no changes in the vascular structures on the iris (Figure 3C and D).

Iridocorneal Angle Angiography

Vessel images appeared at approximately 20 to 23 seconds after the injection with both FA and IA in 2 eyes with iridocorneal angle angiography (patients 5 and 6). In the early stages of FA and IA, the dye perfused several neovascular trunks arising from the iris root. The dye then spread horizontally to perfuse neovascular structures in the trabecular meshwork. Fluorescein angiography showed the bright zonal structure soon after the dye filling because of leakage from the NFVs, and IA showed the structure of the neovascular network because no leakage was observed (Figure 4A and B).

Dye leakage from NFVs markedly decreased with FA after the IV bevacizumab injection, whereas there were no changes in the structure of the vessels observed with IA (Figure 4C and D).

Intraocular Pressure

The IOP decreased after IV bevacizumab injection in 3 of 4 eyes with iris and iridocorneal angle neovascularization (patient 3) and NVG (patients 5 and 6), although 1 patient with NVG (patient 4) did not exhibit decreased IOP. In addition, the patient with NVG (patient 6) was able to halt oral acetazolamide therapy after IV bevacizumab injection. Four eyes were treated with panretinal photocoagulation after the second angiography. One eye (patient 5) underwent a trabeculectomy with mitomycin to control IOP, which increased 3 months after the injection. Another eye (patient 6) received IV bevacizumab again because the IOP increased 4 months after the injection. The clinical data of the patients after IV bevacizumab injection are shown in Table 2.

This study clinically examined the effects of IV bevacizumab injection on the NFVs in the iris and the iridocorneal angle using anterior segment angiography. Fluorescein angiography and IA showed vessels on the pupillary margin and surface of the iris in the eyes with NFVs in the iris. Fluorescein angiography, but not IA, showed leakage of the dye from the pupillary margin and iris surface, whereas the control eye without NFVs showed no leakage with FA. Patients who had eyes with neovascularization in the trabecular meshwork exhibited vascular structures with IA and marked leakage with FA, in clear contrast to the normal eye in which neither IA nor FA demonstrated any vascular structures. Newly formed vessels are associated with endothelial fenestrations through which fluorescein, but not indocyanine green, can pass. The fenestrations are not observed in original iris vessels, as shown by a previous study using scanning laser ophtalmoscopy.17 The vascular structures showing leakage with FA in the iris and iridocorneal angle are believed to represent NFVs.

In the present study, the slitlamp and gonioscopic examinations showed a regression of the NFVs after the IV bevacizumab injection. However, IA showed the same vascular structures in the iris and iridocorneal angle as those before the injection, although the leakage of fluorescein dye from both the iris and the iridocorneal angle vessels markedly decreased after the injection. Indocyanine green angiography is suitable for evaluating the detailed structure of NFVs because of the absence of extravasation of indocyanine green, whereas FA can identify NFVs because of its excellent permeability from the NFVs but not from the original vessels. Therefore, the current results demonstrate that bevacizumab does not reduce NFVs but does reduce the number of endothelial fenestrations, thereby decreasing leakage from the vessels. Kubota et al18 reported in a histopathological study that IV bevacizumab injection reduces serum leakage from the vessels mainly by decreasing the number of endothelial fenestrations in NFVs. Peters et al19 reported that IV

\[\text{Figure 2. Slitlamp and gonioscopic photography of 2 patients with neovascular glaucoma (NVG). In a slitlamp photograph before intravitreal injection of bevacizumab in the NVG eye of patient 4, many newly formed vessels (NFVs) are seen in the margin of the pupil (A), whereas, after bevacizumab treatment, no NFVs are observed in the iris (B). Gonioscopic photograph before bevacizumab treatment in the NVG eye of patient 5 shows NFVs in the superior region of the iridocorneal angle (C), whereas no NFVs were observed in the iridocorneal angle after bevacizumab injection (D).}\]
bevacizumab injection reduced fenestrations of the choriocapillary in a nonhuman primate model. They also showed that bevacizumab significantly reduced vascular endothelial growth factor–induced permeability in cultured porcine choroidal endothelial cells.20 Previous studies21-23 have reported that FA shows substantial reductions in iris neovascularization with minimal leakage in NVG after IV bevacizumab injection. However, it is almost impossible to evaluate the clear vascular structure in the iris by FA because of early and abundant leakage of the dye. Therefore, this decrease in dye leakage may indicate a reduction in NFVs.

Although the mechanism of IOP elevation in NVG with an open angle remains unclear, some authors believe that intertrabecular neovascularization might obstruct the normal aqueous humor outflow,2 whereas others suggest that a high concentration of the serum leaking from the NFVs in the aqueous humor could increase the aqueous outflow resistance.24 In the present study, the IOP showed a decrease in 3 of 4 eyes after IV bevacizumab injection. Previous studies13 reported that IV bevacizumab injection leads to a reduction of the IOP in eyes with early-stage NVG. Three of 4 eyes in the present series exhibited IOPs within the reference range after IV bevacizumab injection, when dye leakage was minimal with FA. These findings suggest that IOP elevation in NVG with an open angle is caused by a high concentration of serum in the aqueous humor owing to increased permeability of the NFVs. Given these findings, we conclude that bevaciz-

Figure 3. Fluorescein (FA) and indocyanine green angiography (IA) of the iris in an eye with neovascular glaucoma (patient 4). At 59 seconds before intravitreal injection of bevacizumab, FA shows remarkable leakage from the newly formed vessels (NFVs) at the pupillary margin and the surface of the iris (A), whereas IA shows no leakage from NFVs despite revealing their structure (B). At 59 seconds after bevacizumab treatment in the same eye, FA shows less dye leakage from NFVs compared with pretreatment (C), whereas IA shows no changes in the images and structures of NFVs (D).
umab reduces vascular permeability without eliminating neovascularization.

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Figure 4. Fluorescein (FA) and indocyanine green angiography (IA) of the iridocorneal angle in an eye with neovascular glaucoma (patient 5). At 60 seconds before intravitreal injection of bevacizumab, FA shows the bright zonal structure with leakage from newly formed vessels (NFVs) (A), and IA shows the structure of the neovascular network (B). At 60 seconds after bevacizumab treatment in the same eye, FA shows less dye leakage from NFVs compared with pretreatment (D), whereas IA shows no change in the images and structures of the neovascular network (D).

Table 2. Clinical Data of Patients With Neovascular Glaucoma After Intravitreal Bevacizumab Injection

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<th>Patient No.</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
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<tbody>
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<td>25</td>
<td>21</td>
<td>10</td>
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<tr>
<td>No. of medications</td>
<td></td>
<td></td>
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<tr>
<td>Eye drops</td>
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<td>NV of iris on slitlamp examination</td>
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Gonioscopic examination results

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<tbody>
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<td>NV of iridocorneal anglea</td>
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<td>ND</td>
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<td>0</td>
</tr>
<tr>
<td>PAS indexa</td>
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<td>ND</td>
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<tr>
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<td>PRP</td>
<td>PRP</td>
<td>PRP, mitomycin + TLC</td>
<td>PRP, bevacizumab</td>
</tr>
</tbody>
</table>

Abbreviations: IOP, intraocular pressure; ND, not done; NV, neovascularization; PAS, peripheral anterior synechia; PRP, panretinal photocoagulation; TLC, trabeculectomy.

a Indicates number of quadrants of the iridocorneal angle with NV or PAS.
Several weeks of active work with Major Smith forced home upon me the conviction that, in his poorly equipped hospital amid the Punjab plains, in the far north of India, he has developed a method of extracting cataract which when patiently studied and mastered by others, as it has been by him will prove a great boon to humanity.\(^3\)

Dr Hermann Knapp [1832-1911, the first editor of the Archives] some years ago made the statement that if Major Smith could perfect his operation for the extraction of the lens in its capsule he would render a greater service to humanity than that rendered by the great Daviel. [Daviel, who lived from 1696-1762, performed the first planned cataract extraction in 1747.]