Bevacizumab (Avastin; Genentech Inc., San Francisco, CA) is a humanized anti-VEGF monoclonal antibody that has been used systemically to treat patients with cancer.[5] For the eye, an intravitreal injection of bevacizumab was found to be effective in reducing the severity of ocular diseases such as neovascular age-related macular degeneration,[6] retinal vein occlusion,[7] and diabetic retinopathy.[8] Bevacizumab has also been used as a preoperative adjunctive therapy for proliferative diabetic retinopathy (PDR).[9] Sawada et al[10] have reported a marked decrease of ocular unbound VEGF level after an intravitreal injection of bevacizumab in eyes with PDR suggesting that the effectiveness of bevacizumab was due to a reduction of the unbound VEGF level.

ROP is a major cause of serious visual impairment in infants born prematurely, and the number of cases of severe ROP is increasing with the increase in the survival rate of the smallest pronatis.[11] In most cases, retinal photocoagulation is very effective in treating eyes with ROP, and the photocoagulation leads to a quiescent stage. However, despite this treatment, some eyes progress to the advanced stages of ROP with proliferative membranes and RDs. For severe cases, vitrectomy must be performed to reattach the retina, although surgeons must wait for the neovascular membranes to become quiescent, which greatly hinders the prognosis of good vision.

VEGF plays a key role in progression of ROPs, and Chung et al[12] have reported that bevacizumab was effective in treating eyes with ROP. This finding suggested that preoperative bevacizumab can be an effective adjunctive therapy for ROP. To the best of our knowledge, there have been only two studies on the ocular VEGF level in eyes with ROP,[13,14] and neither of these reported the level of VEGF after an intravitreal injection of bevacizumab.

Thus, the purpose of this study was to measure the concentration of VEGF in the aqueous humor in eyes with Stage 4 and Stage 5 ROP before and after an intravitreal injection of bevacizumab.

Methods

Subjects

Approval for this study was obtained from the Institutional Review Board of Nagoya University Hospital, and an informed consent was obtained from the parents. The procedures used in this study conformed to the tenets of the Declaration of Helsinki.

Seven eyes at Stage 4 and three at Stage 5 of eight patients with ROP were studied (Table 1). The mean postmenstrual age of the patients was 41.1 weeks (35–64 weeks). Bevacizumab at a dosage of 0.75 mg/0.03 mL was injected intravitreally in 6 eyes of 6 patients. An encircling or buckling procedure was performed on four eyes at Stage 4 ROP. Vitrectomy with lensectomy was performed on two Stage 4B and on two Stage 5 ROP eyes after bevacizumab injection, and in one Stage 5 ROP eye without an injection.

Aqueous humor was collected just before the surgery from seven eyes at Stage 4 and three at Stage 5 eyes. Aqueous humor was also collected just before the intravitreal injection of bevacizumab from two eyes at Stage 4 and two at Stage 5. For control, aqueous humor was also collected from three eyes with congenital cataract and one eye with persistent pupillary membrane that underwent surgery (1 male and 3 female infants). The mean age of these patients was 4.0 ± 2.1 months with a range of 2 to 7 months. Ophthalmoscopy showed that the fundus was normal in these four eyes. Although the eyes used as control were younger than that used in a previous report,[14] they were still older than the ROP eyes. This difference in the ages might have altered the VEGF level.

Ophthalmologic Examinations and Staging of Retinopathy of Prematurity

Fundus and 15-MHz ultrasound biomicroscopy (RION Inc., Kokubunji, Tokyo) examinations were performed on all eyes in the outpatient clinic. Color fundus photographs were taken with RetCam (Massie Research Laboratories Inc., Dublin, CA). Fluorescein angiography was performed under general anesthesia using the fluorescein angiography unit of the RetCam just before the sample collection.

The stage of the ROP was classified according to international classification,[15] and the vascular activity was classified as active if the eye had 1) plus disease, 2) new vessels growing into the vitreous at the ridge of a tractional RD area, or 3) combined effusive and tractional RD.[14]

Sample Collection and Measurement of Vascular Endothelial Growth Factor

Aqueous humor was collected under general anesthesia with a 27-gauge needle just before the surgery or intravitreal injection of bevacizumab. The amount of undiluted aqueous humor collected ranged from 0.02 mL to 0.1 mL. The samples were not analyzed at the time of collection, but were stored in a deep freezer at −80°C until use. The concentration of VEGF was measured by enzyme-linked immunosorbent assay using a commercially available kit (Quantikine: R&D Systems Inc., Minneapolis, MN), which measures both human $VEGF_{121}$ and $VEGF_{165}$. There

Table 1. Patient Characteristics and the Concentration of VEGF in Aqueous Humor

Patient No.	Gestational Age (Weeks)	Postmenstrual Age (Weeks)	Age (Months)	VEGF Before or Without Bevacizumab (pg/mL)	VEGF After Bevacizumab (pg/mL)
Control					
1			2	156	
2			2	ND	
3			5	ND	
4			7	158	
Stage 4 ROP					
1	22	39		564	
2	26	37		944	
3R	27	37		1,750	
3L	27	37		1,890	
4*	24	64		184	
5	25	41†			60 (4 days‡)
6	22	35, 36†		395	ND (4 days‡)
Stage 5 ROP					
7	28	40, 41†		1,990	230 (7 days‡)
8L	23	36, 43†		5,050	290 (48 days‡)
8R*	23	45		370	

*Inactive.
†Postmenstrual age when aqueous humor was collected after bevacizumab injection.
‡Days after bevacizumab injection.
ND, not detectable.

were three samples in which the VEGF was not detectable. However, as the amount of the samples collected from each eye was different and less than 0.2 mL, the minimum amount necessary for the test, all of the samples had to be diluted with Calibrator Diluent RD6U before use. The sample from Control 2 was diluted 10×, Control 3 was diluted 4×, and ROP 6 after bevacizumab was diluted 5× before the measurement. Thus, the concentration of VEGF in these eyes, in which VEGF was not detectable, might have been higher than 31 pg/mL, the minimum detectable limit of this kit.

Results

The concentration of VEGF in the aqueous humor of 1 of the eyes with congenital cataract was 158 pg/mL, and it was less than the detection level in the other 2 eyes. The concentration in an eye with a persistent pupillary membrane was 156 pg/mL.

The concentration of VEGF in 10 eyes with ROP ranged from 184 to 5,050 pg/mL, which is approximately 1.2× to 32× higher than that in the control eyes (Figure 1). Aqueous humor was collected from seven eyes at Stage 4 and three eyes at Stage 5. One eye at Stage 4 and one at Stage 5 were vascularly inactive. The mean concentration of VEGF in the vascularly active ROP was 1,109 pg/mL in the 5 Stage 4 eyes (395, 564, 944, 1,750, and 1,890 pg/mL in the Stage 4 eyes), and 3,520 pg/mL in the 2 Stage 5 eyes (1,990 and 5,050 pg/mL). In the vascularly inactive eyes, the VEGF level was 184 pg/mL in the 1 Stage 4 eye and 370 pg/mL in 1 Stage 5 eye. Thus, the concentration of VEGF in the vascularly active eyes tended to be higher than in inactive eyes, although statistical analysis could not be performed due to the small number of eyes (Figure 1).

Bevacizumab was injected into six active ROP eyes, four at Stage 4 and two at Stage 5, and aqueous humor was collected from two Stage 4 and two Stage 5 eyes 4 to 48 days after the injection just before vitrectomy. In the Stage 4 eyes, the concentration of

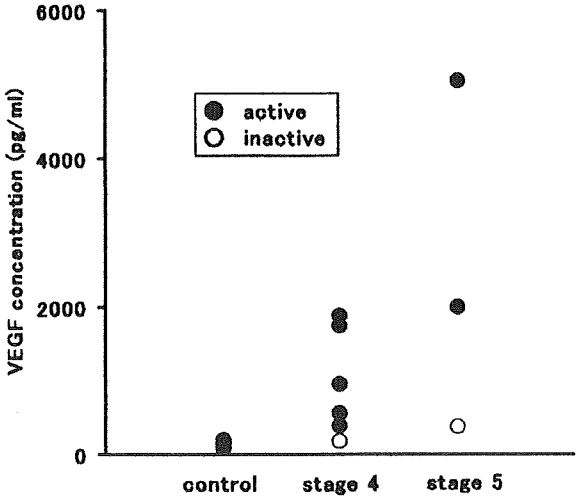

Fig. 1. Concentration of vascular endothelial growth factor (VEGF) in the aqueous humor without or before bevacizumab injection in Stages 4 and 5 ROP and control eyes are shown.

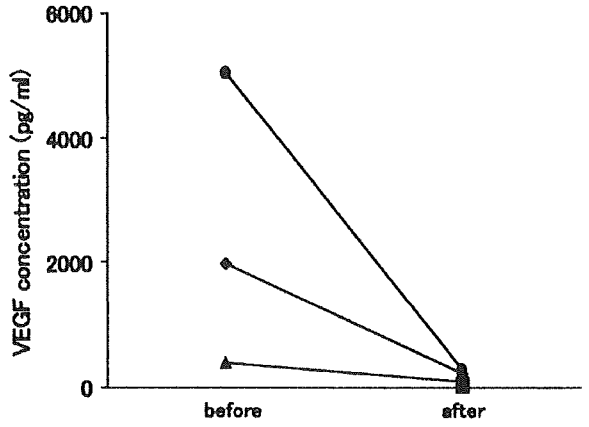

Fig. 2. Vascular endothelial growth factor (VEGF) in the aqueous humor before and/or after bevacizumab injection. The concentration of VEGF in four ROP eyes that had a bevacizumab injection is shown. The concentration before (right) and after (left) injection was measured in three eyes, and after injection in one eye.

unbound VEGF was not detected in 1 eye, and was 60 pg/mL in the other eye 4 days after the injection. In 1 stage 5 eye, the concentration decreased to 230 pg/mL, 7 days after the injection. For 1 Stage 5 eye, vitrectomy was postponed because the patient developed chronic lung disease, and vitrectomy could be safely performed 48 days after the bevacizumab injection. The concentration of VEGF at that time was 290 pg/mL (Figure 2). Fourteen days later, vitrectomy was also performed on the fellow eye without bevacizumab injection, and the concentration of VEGF was 370 pg/mL. The concentration of the VEGF after bevacizumab injection was significantly lower than that of active ROP eyes (145 versus 1,460; $P = 0.04$ with Mann–Whitney U test. The level of VEGF in nondetectable samples was set to 31 pg/mL for statistical analysis). The concentration of VEGF after bevacizumab injection was not measured in two eyes, because the retina was reattached by scleral buckling that was performed together with the injection of bevacizumab, and vitrectomy was not necessary.

Fluorescein angiography was performed before and after the intravitreal injection of bevacizumab in two eyes. In these eyes, there was a considerable decrease of fluorescein leakage from the new vessels after the injection of bevacizumab (Figure 3A and B).

All of the Stage 4 eyes injected with bevacizumab were Stage 4B, and the retina of the two eyes was reattached with one or two vitrectomies without silicon oil tamponade (Figure 3C–F), and two eyes with single encircling surgery (Figure 3G and H). One Stage 5 eye underwent vitrectomy 48 days after the injection, and the retina was reattached under silicon oil (Figure 3I and J). However, one Stage 5 eye with multiple retinal breaks required three surgeries for the retina to be reattached under silicon oil. The silicon oil removal is still being considered for these two eyes.

No ocular complications, such as endophthalmitis, new retinal breaks, or any obvious systemic side effects that were related to bevacizumab were observed.

Comments

The concentration of VEGF in the aqueous humor of eyes at advanced stages of ROP was very high and was higher than that of eyes of adults with PDR (1,460 versus 524; $P = 0.01$ with Mann–Whitney U test) and eyes of infants <1 year without retinal disorders (1,460 versus 94; $P = 0.01$ with Mann–Whitney U test). In these statistical analyses, samples with nondetectable levels of VEGF were set to 31 pg/mL.

Because the concentration of VEGF in the aqueous humor was correlated with the grade of diabetic retinopathy,[16] it would be interesting to know whether there was also a correlation between the VEGF concentration and stage of ROP. Our results showed that the mean VEGF concentration in the Stage 5 eyes was 3× higher than that in the Stage 4 eyes, and one eye at Stage 5 had a level of 5,050 pg/mL. Only three eyes at Stage 4 had <1,000 pg/mL of VEGF. However, Lashkari et al[13] reported that the concentration of VEGF in the subretinal fluid in Stage 4 eyes did not differ significantly from that of Stage 5 ROP eyes. This difference from our results was probably because of the difference in the site where the sample was collected, viz, the aqueous humor or the subretinal fluid. Another reason that might account for this difference might be whether the vessels were active or inactive. Lashkari et al did not report the vascular status of their eye, and it may have been at the vascularly inactive ROP stage. Sonmez et al[14] classified Stage 4 ROP into vascularly active or vascularly inactive state, and reported that the level of VEGF was higher in the active group than in the inactive group as we have found. However, the number of eyes tested in our study was limited, and statistical analysis was not performed. Thus, we cannot make a conclusion on this question.

The intravitreal injection of bevacizumab decreased the concentration of VEGF in the aqueous humor of Stage 4 and Stage 5 ROP eyes markedly, and the concentration decreased to 60, 230, and 290 pg/mL and the level was not detectable in one eye (direct statistical comparison, before and after injection, was not performed because of the small number of the eyes), which is comparable with that of the control eyes of infants. However, it was still higher than that in eyes with PDR after bevacizumab injections reported previously.[10] The concentration in all of these

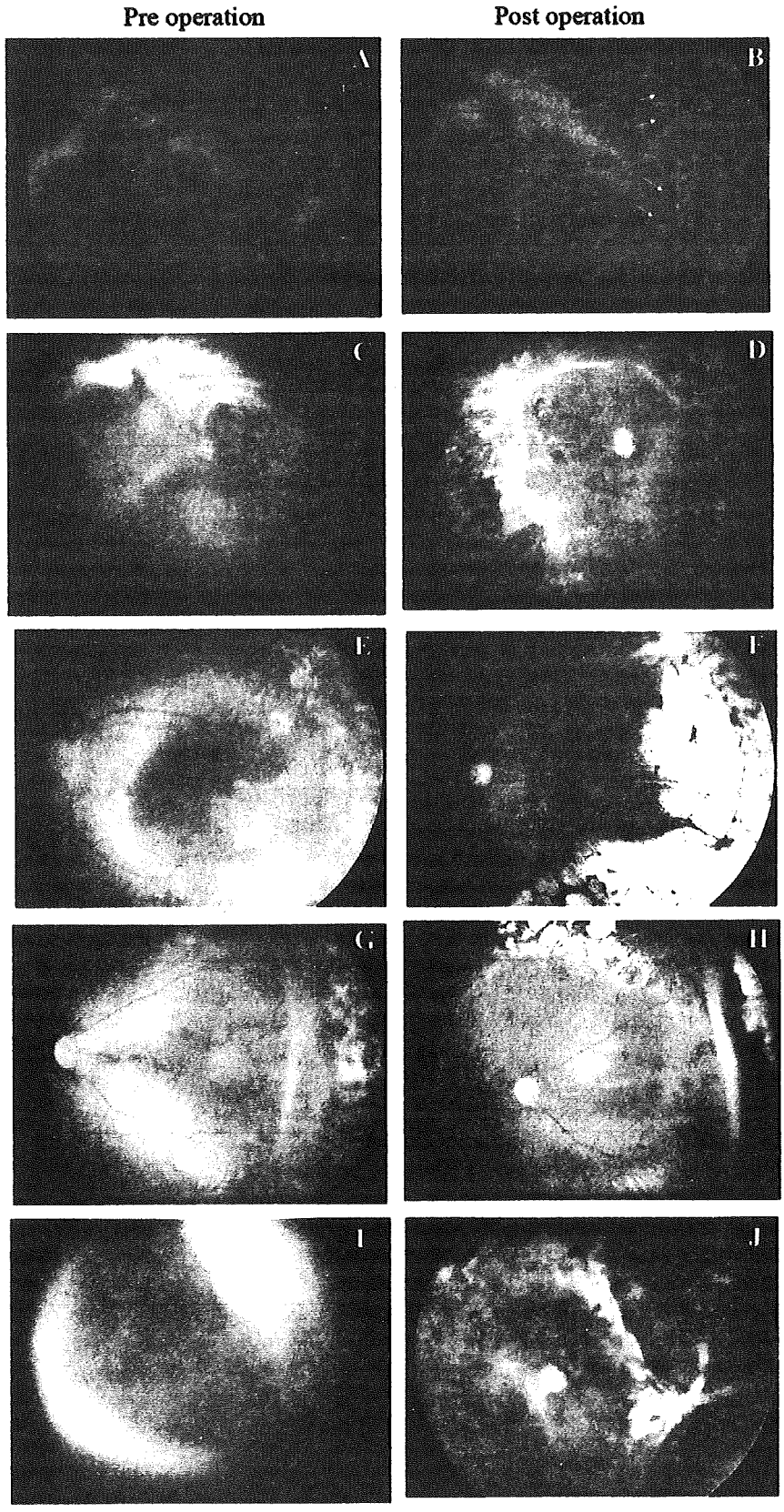

Fig. 3. Clinical outcome of the eyes treated with bevacizumab. (**A–D**) Patient 5 (Stage 4, Table 1). Retina was totally detached except the peripheral retina (**A** and **C**) and bevacizumab was injected as a preoperative adjunctive therapy at postmenstrual age of 40 weeks. Four days after injection, decrease of fluorescence leakage (arrow) was observed (**B**) and vitrectomy with lensectomy was performed. One month later, second membrane removal operation was performed, and retina was totally reattached (**D**). The visual acuity of this eye is LS (+) 1 year after the second surgery may be due to the good vision of the fellow eye, and the vision training is performed. (**E** and **F**) Patient 6 (Stage 4, Table 1). Because of the strong proliferation of active new vessels, bevacizumab was injected as preoperative adjunctive therapy at the postmenstrual age of 35 week (**E**). Four days after injection, vitrectomy with lensectomy was performed, and the retina was totally reattached. Four months after operation, macular is formed (**F**) and vision tracking is obtained. (**G** and **H**) Patient 2 (Stage 4, Table 1). Encircling surgery was performed at postmenstrual age of 35 weeks, but as the vascular activity was high and the retinal detachment remained (**G**), vitrectomy was planned. However, after the bevacizumab injection at 14 days after the encircling procedure, the progression stopped and the retina was reattached. Six months after the injection, the macular configuration was present (**H**) and visual acuity of 0.75 cycles/degree (Teller Acuity Cards) was obtained. (**I** and **J**): Patient 8L (Stage 5, Table 1). At postmenstrual age of 36 weeks, the retina was totally detached to closed funnel shape with multiple retinal breaks (**I**). Bevacizumab was injected, but vitrectomy was postponed because of the chronic lung disease. Vitrectomy with lensectomy was performed 48 days after the bevacizumab injection at the postmenstrual age of 43 weeks, and silicon oil was injected because of the retinal break. One year after the operation, the retina is reattached (**J**), and silicone oil removal is considered.

eyes was below the detection level of 31 pg/mL (no statistical analysis was performed). This difference might be because the VEGF concentration before the injection was much higher in the eyes with ROP, or the amount of bevacizumab injected in PDR eyes was greater. However, because the assays were run by different methods, at different institutes, and at different times, it might not be proper to compare these results. Although there was detectable VEGF remaining in some eyes, it was decided not to increase the amount of bevacizumab for these infants because we did observe a decrease of fluorescein leakage after the injection and less bleeding during vitrectomy with this dosage, and because of the possible systemic side effects. It also is possible that the residual level of VEGF might have prevented the topical side effects, e.g., obstruction of the development of normal vessels and neural retina, as the level is similar to that of control eyes, although the distribution of VEGF might be different from normal.

One Stage 5 eye became vascularly inactive and its VEGF level was 370 pg/mL, 62 days after the bevacizumab was injected into the fellow eye. However, it is not clear whether this was due to circulating bevacizumab or just a natural course of ROP. Because there was no ROP eye in which samples were collected twice in 4 or 7 days without bevacizumab as control, there is a possibility that decrease in ocular VEGF in other bevacizumab injected eyes can be the natural course of this disease. However, as a rapid inactivation of ROP was observed in these eyes and bevacizumab is shown to decrease VEGF in other diseases such as PDR,[10] this rapid decrease of VEGF is likely to be the effect of this drug.

From August 2004 to November 2006, vitrectomy was performed for Stage 4B (3 eyes) and Stage 5 (17 eyes) eyes without bevacizumab injection at our hospital. The average postmenstrual age of these infants was 49.7 weeks (38–67 weeks). Without bevacizumab, vitrectomy was performed 2.1 ± 0.9 times/eye (1–5 times), and the rate of reattachment was 55%. However, vitrectomy could be performed on the eyes treated with bevacizumab without severe bleeding during membrane removal at postmenstrual age of 40.3 weeks (36–43 weeks) which is earlier than that without bevacizumab, without severe bleeding during membrane removal. In two Stage 4B eyes, reattachment of the retina was obtained without silicon oil tamponade with one or two surgeries, and one Stage 5 eye, reattachment of the retina under silicon oil was obtained with one surgery. The removal of the silicon oil is being considered. For one Stage 5 eye with multiple retinal breaks, reattachment of the retina under silicon oil was obtained with three surgeries, and removal of the silicon oil is still being considered. Moreover, two Stage 4B eyes, which received an injection of bevacizumab just before or 2 weeks after encircling surgery, had a reattachment of the retina without additional operations. From August 2004 to November 2006, encircling surgery was performed without bevacizumab at our hospital for two Stage 4B eyes, but both eyes progressed to Stage 5, and vitrectomy was required for reattachment. These results suggest that bevacizumab injection might be useful adjunctive therapy for both vitrectomy and encircling surgery for severe ROP, although the number of the eyes studied was very limited.

In conclusion, an intravitreal injection of bevacizumab decreased the VEGF level markedly in the aqueous humor of ROP eyes although a direct comparison, before and after injection, was not performed statistically. However, the concentration of VEGF was not below the detection level in most of the eyes after injection. Our findings indicate that bevacizumab might be useful for preoperative adjunctive therapy for ROP, however the number of eyes studied was very limited and further studies are needed.

Key words: retinopathy of prematurity, vascular endothelial growth factor, bevacizumab, intravitreal injection, aqueous humor.

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Microincision Vitrectomy Surgery and Intravitreal Bevacizumab as a Surgical Adjunct to Treat Diabetic Traction Retinal Detachment

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Purpose: To investigate the feasibility and efficacy of microincision vitrectomy surgery (MIVS) combined with intravitreal bevacizumab (IVB) as a surgical adjunct for treating traction retinal detachment (TRD) secondary to severe proliferative diabetic retinopathy (PDR).

Design: Retrospective, comparative, consecutive, interventional case series.

Participants: Seventy-one eyes of 59 consecutive patients who underwent primary vitrectomy for diabetic TRD and were followed up for more than 6 months after surgery.

Methods: Eyes that received IVB (1 mg) as a preoperative adjunct followed by MIVS (IVB/MIVS group) from November 2005 through December 2007 were compared with eyes that underwent conventional 20-gauge pars plana vitrectomy (20-g PPV group) from September 2003 through October 2005.

Main Outcome Measures: Primary and ultimate anatomic success, intraoperative and postoperative complications, and final visual success with at least 6 months of follow-up.

Results: This series included 38 eyes (33 patients) in the IVB/MIVS group and 33 eyes (26 patients) in the 20-g PPV group. The primary and ultimate anatomic success rates (95% vs. 91% and 100% in both groups, respectively) and the mean visual acuity changes did not differ significantly between groups; the surgical time and intraoperative bleeding in the IVB/MIVS group decreased significantly compared with the 20-g PPV group ($P<0.001$). The rate of visual improvement of 3 lines or more at the 6-month follow-up was 68% in the IVB/MIVS group and 49% in the 20-g PPV group, respectively. Progression of the preexisting TRD after IVB occurred in 7 eyes (18%). Absence of previous laser photocoagulation ($P = 0.025$) and the presence of a ring-shaped fibrovascular membrane ($P = 0.013$) were relevant findings in eyes with these IVB-induced complications.

Conclusions: Intravitreal bevacizumab plus MIVS offers comparable anatomic success compared with conventional 20-gauge PPV in patients with TRD resulting from severe PDR. This technique shortens the surgical time with fewer intraoperative complications and favorable visual recovery. However, caution should be taken because of rapid progression of the preexisting TRD after IVB in some patients.

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Tractional retinal detachment (TRD) is a serious complication of severe proliferative diabetic retinopathy (PDR). Conventional 20-gauge pars plana vitrectomy (PPV) is the standard treatment of choice for PDR and achieves good anatomic success.[1–5] However, surgically induced complications, that is, intraoperative bleeding and iatrogenic retinal breaks, recurrent or persistent vitreous hemorrhage, or both, retinal redetachment, postoperative reproliferation, anterior hyaloid fibrovascular proliferation, and development of neovascular glaucoma (NVG) are major concerns in diabetic vitrectomy,[6–11] sometimes resulting in poor anatomic and serious long-term visual impairment.[12,13]

Recent advances in surgical and medical retina treatment, that is, microincision vitrectomy surgery (MIVS) and intravitreal injection of drugs targeting vascular endothelial growth factor (VEGF), are impressive options for treating a variety of vitreoretinal diseases. This trend toward less invasive therapy led the authors to evaluate the synergistic effect of combining the 2 approaches to treat complications of severe PDR.

Transconjunctival MIVS with 25- or 23-gauge instrumentation has several advantages over traditional 20-gauge vitrectomy.[14–19] Smaller incisions without peritomy and self-sealing sclerotomy may decrease trauma with faster wound healing, may diminish conjunctival scarring and patient discomfort, and may decrease postoperative inflammation and induced astigmatism, theoretically facilitating early visual recovery in macular diseases.[20–23] Although

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MIVS initially was recommended for macular pathologic features or simple vitreous hemorrhage because of the limited designs and fragility of small-gauge instrumentation in the initial stage, recent development of rigid instrumentation and a bright light source expanded the indications for MIVS to include complex vitreoretinal disorders such as rhegmatogenous retinal detachment (RRD) and proliferative vitreoretinal disorders.[16,18,19,24,25] Transconjunctival MIVS should be more suitable than traditional 20-gauge PPV for treating severe PDR because the conjunctiva-preserving nature of MIVS allows repeated vitrectomy or filtering surgery that may be needed in patients with diabetes complicated with NVG even after vitrectomy. Less postoperative inflammation associated with MIVS may facilitate early visual recovery in PDR as well as in macular diseases. However, it still remains a concern whether complex intraocular manipulations such as fibrovascular membrane dissection and hemostasis in diabetic vitrectomy can be managed steadily using small-gauge instrumentation.

Intraocular neovascularization in patients with diabetes is correlated with retinal ischemia, which stimulates VEGF, a key molecule responsible for ocular neovascularization.[26,27] Direct targeting of VEGF with anti-VEGF pharmacotherapy is another therapeutic option for intraocular neovascularization.[28,29] Bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA), a full-length humanized monoclonal antibody that binds all VEGF isoforms, was approved by the Food and Drug Administration for treating metastatic colorectal cancer.[30] Recent studies of off-label intravitreal bevacizumab (IVB) injections have reported the efficacy and safety of bevacizumab for preventing intraocular bleeding and postoperative complications in standard 20-gauge vitrectomy or filtering surgery.[31–35] Therefore, IVB as a surgical adjunct to MIVS is expected to reduce concerns about intraoperative bleeding and to encourage the use of MIVS for complications associated with severe PDR.

Adjunctive IVB was used to suppress intraocular neovascularization followed by MIVS in consecutive patients with TRD secondary to severe PDR. The purpose of this study was to evaluate retrospectively the feasibility and outcomes of this approach compared with traditional 20-gauge vitrectomy without preoperative IVB.

Patients and Methods

Inclusion and Exclusion Criteria

This retrospective chart review included a consecutive series of patients with diabetes who underwent primary PPV for TRD secondary to PDR between September 2003 and December 2007 at Osaka University Medical School Hospital or Kagawa University Hospital. The institutional review boards of both facilities approved the study. Patients provided written informed consent after they received a detailed description of the surgical procedure. The study adhered to the tenets of the Declaration of Helsinki.

Patients had not undergone previous vitreoretinal surgery except laser photocoagulation to treat PDR or clinically relevant diabetic macular edema. The indications for PPV included a TRD involving the macula, combined TRD and RRD, or extramacular TRD with an adherent fibrovascular membrane causing excessive macular traction. The macular status and the extent of the TRD were confirmed by B-scan echography in patients with a preretinal hemorrhage that obscured clear fundus visualization. Patients were excluded if they had a TRD for more than 1 year and a best-corrected visual acuity of less than hand movements, an extramacular TRD without macular traction, or less than 6 months of follow-up after the initial surgery.

Surgical Technique

From September 2003 through October 2005, PPV was performed using a standard 20-gauge 3-port system (20-gauge PPV group). After conjunctival peritomy, sclerotomies were introduced using a 20-gauge microvitreoretinal blade. Core vitrectomy using a high-speed vitreous cutter (2500 cycles/minute) was performed with intravitreal injection of triamcinolone acetonide to visualize the vitreous gel and to identify the location of vitreoretinal adhesions. Peripheral vitrectomy was performed to relieve anterior posterior traction. Fibrovascular membrane dissection and segmentation were performed to remove all tangential traction using a magnifying prismatic lens. Delamination techniques also removed as much fibrovascular tissue as possible with vitreorctinal scissors, forceps, and a tissue manipulator. Vitreous base shaving (360°) under scleral depression was performed to remove peripheral cortical gel and release epicenters of anterior fibrovascular proliferation. Endolaser photocoagulation was applied to complete panretinal photocoagulation up to the anterior retina. Any retinal breaks also were treated. In pseudophakic eyes, posterior capsulectomies were created using the vitreous cutter to improve intraocular visualization if needed. Phacoemulsification and aspiration were performed simultaneously through a 3.0-mm sclerocorneal wound in patients with cataract. An acrylic foldable intraocular lens (IOL) was placed in the capsular bag in patients with an intact capsule. Fluid–gas exchange was performed for retinal breaks, RRD, or both, by flushing with 50 ml of a premixed nonexpansile concentration of 20% sulfur hexafluoride or 14% perfluoropropane. In patients with severe visual impairment in the fellow eye, silicone oil tamponade was considered. After surgery, all sclerotomies and conjunctival incisions were sutured and steroid was injected subconjunctivally. Topical antibiotic ointment was applied, and the eye was patched and shielded. Patients who had undergone fluid–gas exchange were instructed to remain face down for 7 to 10 days.

From November 2005 through December 2007, diabetic TRD was treated by MIVS after adjunctive pretreatment with IVB administered (IVB/MIVS group) using strict aseptic techniques.[36] A 50-μl aliquot of commercially available bevacizumab (25 mg/ml) was prepared and placed in a tuberculin syringe with a 29-gauge needle and was refrigerated until use. Bevacizumab (1 mg) was injected intravitreally via the pars plana. In patients with increased IOP, a paracentesis (100–200 μl) was created to normalize the IOP. Patients were instructed to use topical levofloxacin or gatifloxacin and were reexamined on days 1, 3, and every week after injection. During the follow-up visit, visual acuity (VA) and IOP measurements were obtained and slit-lamp microscopy and fundus microscopy were performed. If patients had media opacities that obscured the fundus, the extent and progression of the TRD were evaluated by B-scan echography.

Microincision vitrectomy surgery was performed using either a 25- or 23-gauge trocar or cannula system (Alcon Laboratories, Inc., Fort Worth, TX; Dutch Ophthalmic Research Center, Zuidland, The Netherlands) by no more than 1 month after IVB pretreatment. Surgeon preference determined the gauge without randomization; the standard MIVS procedures have been described previously.[15–19] The trocar and cannula device was inserted using the 2-step entry method developed by Eckardt.[17] After oblique insertion at approximately a 30° to 45° angle to the sclera

of a 25- or 23-gauge microvitreoretinal blade through the conjunctiva and sclera after conjunctival displacement to misalign the conjunctiva and sclera incisions, blunt trocars for transscleral cannulas were inserted through the pars plana in the supranasal, supertemporal, and inferotemporal quadrants for infusion and insertion of intraocular instruments. To overcome the limitations and fragility of small-gauge instrumentation, several surgical techniques and devices have been modified and developed. In contrast to the halogen illumination commonly used for traditional 20-gauge PPV, a xenon or mercury vapor light source is used in MIVS to obtain bright endoillumination and clear visualization.[37,38] The surgical techniques for removing fibrovascular membranes during MIVS differ from those in traditional 20-gauge surgery; that is, the membranes are cut and removed using a small-gauge high-speed vitrectomy cutter instead of vitreoretinal scissors (see Video 1, available at http://aaojournal.org). When fibrovascular membranes extensively adhered to the detached retina, a bimanual technique using a membrane forceps and a vitrectomy cutter was performed using a 25- or 27-gauge self-retaining chandelier illumination fiber[37,38] (see Video 2, available at http://aaojournal.org). A peripheral vitrectomy and vitreous base shaving were performed under a wide-angle viewing system instead of scleral depression.[39,40] Endolaser photocoagulation up to the anterior retina was applied using an extendable laser probe. To preserve the conjunctiva during MIVS, phacoemulsification and IOL implantation were performed through a 3-mm clear corneal incision, and a sutureless contact lens ring was used during vitrectomy.[19] At the end of surgery, the IOP was decreased to identify rebleeding from vascular epicenters, and endoillumination with gentle scleral indentation helped to identify peripheral retinal pathologic features. After removing the supertemporal and supranasal cannulas with the infusion clamped, the clamp–unclamp infusion technique was performed,[11] and the sclerotomies were inspected for fluid leaks. The inferotemporal cannula and infusion line were removed en bloc with the infusion unclamped followed by conjunctival repositioning and inspection. If the vitreous cavity was filled with air or long-acting gas at the end of surgery, air-tightness was confirmed by pouring balanced salt solution (Alcon Laboratories, Inc.) around the wounds to check air bubbling through the sclerotomy. If a bleb leak was found, transconjunctival sutures were placed to close the leaky sclerotomy. The other procedures were the same as the traditional 20-gauge procedures described previously.

Data Analysis

The patient medical records and surgical notes were reviewed for age; gender; follow-up; lens status; previous treatments; TRD type; coexisting complications; systemic data including serum creatinine levels and hemoglobin A1c levels; preoperative best-corrected visual acuity; postoperative best-corrected VA at the 6-month follow-up visit; preoperative and postoperative IOP at the 1-day, 1-week, and 6-month follow-up visits; operating time; anatomic success rate; and intraoperative and postoperative complications. Instrumentation used to dissect and remove fibrovascular membranes, and the strategies for intraoperative hemostasis were recorded by reviewing the surgical video and summary sheets. The severity of intraoperative bleeding was graded based on the methodology for achieving hemostasis, with 0 indicating self-limited bleeding without hemostatic procedures; 1 indicating intraoperative hemostasis via gentle, direct depression of the bleeding point with the blunt tip of a vitreous cutter; 2 indicating hemostasis with elevated IOP; and 3 indicating hemostasis with elevated IOP and cautery of the bleeding point. In patients who underwent adjunctive IVB pretreatment and MIVS, the efficacy and complications of IVB, requirement for conversion to 20-gauge instrumentation during surgery, and the rate of self-sealing also were reviewed. Postoperative hypotony was defined as an IOP of 7 mmHg or less. The VA was measured using the Landolt C acuity chart and was analyzed on a logarithm of minimal angle of resolution (logMAR) scale. Counting fingers vision was defined as 0.01 (2.0 logMAR) and hand movements was defined as 0.001 (3.0 logMAR).[41] Visual improvement was defined as an increase of at least 0.3 logMAR unit. Where appropriate, the Mann–Whitney rank-sum test, the Student t test, and the Fisher exact test were used to compare the differences in the groups. Statistical analysis was performed using SPSS software version 10.0J (SSPS, Inc., Chicago, IL). $P \leq 0.05$ was considered to be statistically significant.

Results

A total of 71 eyes of 59 patients (38 eyes in the IVB/MIVS group and 33 eyes in the 20-gauge PPV group) met the study criteria. The patient demographics and preoperative ocular findings for the 2 groups are listed in Table 1. Although both eyes of 5 patients in the IVB/MIVS group and both eyes of 7 patients in the 20-gauge PPV group met the study criteria and were enrolled for data analysis, there were no significant differences in the baseline parameters between the groups, including age, bilaterality, preoperative VA, lens status, TRD type, preexisting complications, and major systemic data between the groups. The surgical results were compared for the 2 study intervals in which different procedures and instruments were used. The mean follow-up period of 17.5±13.3 months in the 20-gauge PPV group was substantially longer than the mean follow-up of 11.8±4.5 months in the IVB/MIVS group, but the difference did not reach significance ($P = 0.43$). Six-month follow-up data were available for every patient in both groups.

Anatomic Success Rate and Visual Recovery

The initial anatomic success rate was 95% in the IVB/MIVS group and 91% in the 20-gauge PPV group (Table 2). Although vitreoretinal reoperations were required for retinal reattachment, the final anatomic success rate was 100% in both groups. The VA levels in the 71 study eyes at baseline and 6 months after surgery are shown in Figure 1. The preoperative VA ranged from hand movements to 0.7 (mean, 0.03) in the IVB/MIVS group and from hand movements to 0.8 (mean, 0.05) in the 20-gauge PPV group. The mean postoperative VA was 0.30 in the IVB/MIVS group and 0.22 in the 20-gauge PPV group. The mean postoperative VA improved significantly compared with the mean preoperative VA in both groups ($P<0.001$). Despite no significant differences in the mean preoperative VA between the groups ($P = 0.46$), the difference in the mean VAs 6 months after surgery reached borderline significance ($P = 0.047$). However, the overall mean VA changes before and 6 months after surgery (logMAR units, −0.81±1.16 in the IVB/MIVS group vs. −0.47±0.92 in the 20-gauge PPV group) did not differ significantly between the groups ($P = 0.18$). Figure 2 shows that the VA at the 6-month follow-up improved by 3 lines or more in 26 eyes (68%), was unchanged in 8 eyes (21%), and decreased by 3 lines or more in 4 eyes (11%) in the IVB/MIVS group. In the 20-gauge PPV group, the VA improved in 16 eyes (49%), was unchanged in 13 eyes (39%), and decreased in 4 eyes (12%). Visual improvement in the IVB/MIVS group was higher than that in the 20-gauge PPV group, but the difference between groups did not reach to statistical significance ($P = 0.14$).

Surgical Procedures and Intraoperative Complications

The main surgical techniques during vitrectomy were similar for the 2 groups (Table 2). However, the surgical time for IVB/MIVS

Table 1. Patient Demographics and Baseline Ocular Findings

Parameters	Intravitreal Bevacizumab/Microincision Vitrectomy Surgery Group	20-Gauge Pars Plana Vitrectomy Group	P Value
No. eyes/patients	38/33	33/26	
No. men/women	19/14	15/11	0.80
Age (yrs)			
Mean±SD	50.3±14.0	54.5±10.9	0.22
Range	22–73	28–67	
Bilaterality, no. (%)	5 (15)	7 (27)	0.43
Preoperative BCVA			
Mean (range)	0.03 (HM–0.7)	0.05 (HM–0.8)	
LogMAR±SD	1.34±0.95	1.13±0.83	0.46
IOP (mmHg)			
Mean±SD	17.9±9.2	15.6±4.2	0.35
Range	8–55	8–31	
Lens status			
Phakia/pseudophakia	31/7	29/4	0.69
Type of RD, no. (%)			
TRD only	32 (84)	28 (85)	0.80
Combined TRD/RRD	6 (16)	5 (15)	
Macula involved, no. (%)	17 (45)	13 (39)	0.83
PVD, no. (%)			
None	12 (32)	12 (36)	0.86
Incomplete	26 (68)	21 (64)	
Preoperative complication, no. (%)			
Persistent VH	17 (45)	12 (36)	0.63
INV or NVG	5 (13)	6 (18)	0.80
Previous PRP, no. (%)			
None	8 (21)	6 (18)	0.99
Incomplete	30 (79)	27 (82)	
Systemic data			
HbA_{1c} (%)±SD	7.7±1.9	7.3±1.1	0.72
Creatinine (mg/dl)±SD	1.4±1.6	1.2±0.8	0.87
Follow-up (mos)			
Mean±SD	11.8±4.5	17.5±13.3	0.43
Range	6–27	6–47	

BCVA = best-corrected visual acuity; HbA_{1c} = hemoglobin A_{1c}; HM = hand movements; INV = iris neovascularization; IOP = intraocular pressure; logMAR = logarithm of minimal angle of resolution; NVG = neovascular glaucoma; PRP = panretinal photocoagulation; PVD = posterior vitreous detachment; RRD = rhegmatogenous retinal detachment; SD = standard deviation; TRD = traction retinal detachment; VH = vitreous hemorrhage.

was significantly shorter ($P<0.001$) than for conventional 20-gauge PPV. The chance of using microforceps and microscissors to remove fibrovascular membranes during IVB/MIVS was significantly less than during conventional 20-gauge PPV ($P = 0.02$ and $P<0.001$, respectively). Despite extensive peripheral shaving in both groups, 28 eyes (74%) in the IVB/MIVS group had self-sealing sclerotomies without sutures. Conversion to 20-gauge instrumentation to complete surgery was unnecessary in the IVB/MIVS group.

The intraoperative and postoperative complications in both groups are summarized in Table 3. Overall, the incidence of intraoperative iatrogenic retinal tears did not differ significantly between the groups ($P = 0.11$); however, the incidence in the 20-gauge PPV group (41%) was higher than that in the IVB/MIVS group (19%). Sclerotomy-related retinal tears occurred in 2 eyes (6%) in the 20-g PPV group. The other retinal breaks in both groups occurred during posterior manipulations including membrane removal. Iatrogenic tears were treated during surgery by endolaser photocoagulation, cryoretinopexy, or both and were unrelated to subsequent complications such as RRD.

Hemorrhages during fibrovascular membrane manipulation occurred in all cases regardless of the use of conventional 20-gauge or small-gauge instruments. However, the severity of the intraoperative bleeding differed significantly ($P<0.001$) between the groups, suggesting that intraoperative bleeding in the conventional 20-gauge PPV group was more severe and that it was more difficult to achieve hemostasis than in the IVB/MIVS group. Ten (32%) of 31 phakic eyes in the IVB/MIVS group and 7 (24%) of 29 phakic eyes in the conventional 20-g PPV group underwent vitrectomy with crystalline lens sparing. No eyes had crystalline lens-related complications such as inadvertent instrument touch or secondary cataract formation.

Postoperative Complications and Reoperation Rates

The major postoperative complication was transient fibrin formation in the anterior chamber, vitreous cavity, or both in 9 eyes (24%) in the IVB/MIVS group and in 15 eyes (45%) in the 20-gauge PPV group. All fibrin formation resolved after postoperative topical steroid administration and did not affect vision.

Postoperative vitreous hemorrhage developed in 5 eyes (13%) in the IVB/MIVS group and in 9 eyes (27%) in the 20-gauge PPV group. Of these, 1 eye (20%) in the IVB/MIVS group and 4 eyes (44%) in the 20-gauge PPV group underwent repeated vitrectomy, after which no eyes had recurrent postoperative vitreous hemor-

Table 2. Surgical Procedures at the Initial Surgery and Outcomes

	Intravitreal Bevacizumab/ Microincision Vitrectomy Surgery Group (n = 38)	20-Gauge Pars Plana Vitrectomy Group (n = 33)	P Value
Operating time (minutes)	85±47	123±42	<0.001
PEA and IOL, no. (%)*	21 (55)	22 (67)	0.46
Instrumentation for FVM removal, no. (%)†			
Vitreoretinal forceps	23 (61)	29 (88)	0.02
Membrane scissors and/or MPC‡	8 (21)	26 (79)	<0.001
Endolaser photocoagulation	35 (92)	32 (97)	0.62
Tamponade, no. (%)			
Gas (air or long-acting gas)	18 (47)	14 (42)	0.92
Silicone oil	1 (3)	1 (3)	
Self-sealing, no. (%)¶	28 (74)	0	<0.001
Initial reattachment, no. (%)	36 (95)	30 (91)	1.00
Final reattachment, no. (%)	38 (100)	33 (100)	1.00
Postoperative BCVA§			
Mean (range)	0.30 (HM–1.2)	0.22 (HM–1.0)	
LogMAR±SD	0.51±0.71	0.65±0.58	0.047
Mean BCVA changes (logMAR±SD)‖	−0.81±1.16	−0.47±0.92	0.18

BCVA = best-corrected visual acuity; FVM = fibrovascular membrane; HM = hand movements; IVB = intravitreal bevacizumab; logMAR = logarithm of minimal angle of resolution; MPC = automated membrane peeling cutting scissors; PEA and IOL = phacoemulsification and intraocular lens implantation; PPV = pars plana vitrectomy; SD = standard deviation.

*Phacoemulsification and IOL were performed simultaneously with PPV in eyes complicated with preexisting crystalline lens opacification.

†Fibrovascular membrane removal included segmentation, delamination, peeling, and dissection of the membranes for removal from the retina.

‡Membrane scissors were a 20- or 25-gauge instrument; MPC is available only in 20 gauge.

¶Self-sealing is defined as the absence of need for suture placement to any sclerotomies at the end of surgery.

§The postoperative BCVA was measured at the 6-month follow-up visit.

‖Best-corrected visual acuity changes were defined as the changes between the preoperative and the 6-month postoperative BCVA in logMAR.

rhage during follow-up. Anterior hyaloid fibrovascular proliferation[9] did not develop in any eyes throughout the follow-up period.

Iris neovascularization (INV) and NVG are the most serious postoperative complications of vitreous surgery for severe PDR.[10,11] Although no new INV or NVG occurred during follow-up, preexisting INV and NVG were stabilized or controlled in only 2 (40%) of 5 eyes in the IVB/MIVS group and in 4 (67%) of 6 eyes in the 20-gauge PPV group after surgery. Despite the preoperative injection of bevacizumab in the IVB/MIVS group, control of the preexisting INV and NVG after surgery did not differ significantly between groups.

Recurrent retinal detachment complicated by progressive fibrovascular proliferation developed in 2 eyes (5%) in the IVB/MIVS group and in 3 eyes (9%) in the 20-gauge PPV group, but the difference was not significant. An RRD developed in 1 eye (3%) in the 20-gauge PPV group. The causal retinal break was inferonasal but was not associated with sclerotomy-related complications. All 6 eyes underwent another vitrectomy and achieved retinal reattachment. Of the 2 eyes in the IVB/MIVS group, MIVS was repeated with 14% perfluoropropane gas tamponade.

Although self-sealing sclerotomies were achieved in 28 eyes (74%) in the IVB/MIVS group, postoperative hypotony-related maculopathy occurred in 1 eye (3%) in the early study period of MIVS. Because a macular fold persisted even after the IOP returned to normal, repeated vitrectomy with internal limiting membrane peeling and air tamponade was performed and flattened the retinal fold successfully.

Overall, in the IVB/MIVS group, vitreoretinal reoperations were the result of persistent vitreous hemorrhage (n = 1), recurrent retinal detachment (n = 2), and hypotony-related maculopathy (n = 1). In the 20-g PPV group, vitreoretinal reoperations were required because of persistent vitreous hemorrhage (n = 4), recurrent retinal detachment with progressive reproliferation (n = 3), and newly developed RRD after the initial surgery (n = 1). The reoperation rate did not differ significantly between the groups (P = 0.22).

Rapid Progression of Tractional Retinal Detachment after Adjunctive Intravitreal Bevacizumab

The mean interval between IVB and MIVS was 7.6 days (range, 2–30 days). No adverse systemic complications related to IVB occurred during follow-up. Rapid progression of the preexisting TRD occurred in 7 eyes (18%) after IVB. The patient demographics and baseline ocular characteristics with or without rapid progression of the TRD after IVB are shown in Table 4. There were no significant differences in the preoperative VA and IOP, TRD type, preexisting complications such as vitreous hemorrhage and INV/NVG, interval between IVB and MIVS, and major systemic data between eyes with or without rapid progression of TRD after IVB. Preoperative ring-shaped fibrovascular membrane formation (P = 0.013) and absence of previous panretinal photocoagulation (P = 0.025) were significant in eyes with rapidly progressive TRD. The final mean VA in eyes with progression of the preexisting TRD (0.15) was worse compared with eyes without this complication (0.5).

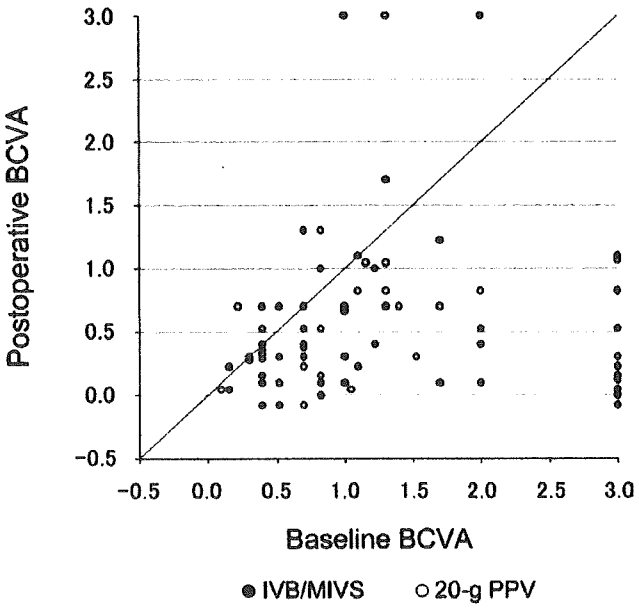

Figure 1. Scatterplot showing the changes in baseline and postoperative best-corrected visual acuity (BCVA) measured at the 6-month follow-up visit in each group. The BCVA was converted to logarithm of the minimum angle of resolution units. Overall, the mean postoperative BCVA improved significantly compared with the mean baseline BCVA in both groups ($P<0.001$). IVB/MIVS = intravitreal bevacizumab-assisted microincision vitrectomy surgery group; 20-g PPV = 20-gauge pars plana vitrectomy group.

Case Reports

Case 1. A 46-year-old man with poorly controlled diabetes underwent bilateral panretinal photocoagulation. Despite extensive treatment, a prominent fibrovascular membrane developed in the left eye causing a macular TRD (Fig 3A). The VA in the left eye was 0.06. Bevacizumab (1 mg) was injected intravitreally in the left eye before MIVS. Four days later, the neovascular components in the fibrovascular membrane regressed with rapid fibrotic changes and reduction in the size of many abnormal new vessels (Fig 3B). Microincision vitrectomy surgery was uneventful with minimal intraoperative bleeding and easy membrane dissection using a 25-gauge vitrectomy (Fig 3C). Six months later, the VA improved to 0.15, and the macula was attached without reproliferation (Fig 3D).

Case 2. A 48-year-old woman with insulin-dependent diabetes mellitus and no previous ocular treatment was referred to the authors. She had bilateral dense cataracts, rubeosis iridis, and a small pupil that obscured the fundus findings. However, large-caliber neovascular vessels comprising diffuse neovascularization elsewhere and a TRD with a large ring-shaped fibrovascular membrane were faintly visible in the left eye (Fig 4A). B-scan echography confirmed a shallow, extensive TRD (Fig 4B). The VA in the left eye was 0.02. Bevacizumab (1 mg) was injected intravitreally into the left eye to hasten regression of INV and retinal neovascularization and to prevent intraoperative bleeding. Four days later, despite INV regression, B-scan echography confirmed progression of the preexisting TRD (Fig 4C). The patient underwent uneventful MIVS, phacoemulsification, and IOL implantation using the 25-gauge system. A total retinal detachment with extensive contraction of the fibrotic membrane was observed during surgery (Fig 4D). The membrane was separated from the retinal surface and was dissected bimanually using membrane forceps and a 25-gauge vitrectomy with slight bleeding from the epicenters (Fig 4E). One month later, focal retinal redetachment with membrane reproliferation was treated successfully by repeated MIVS with the 25-gauge system. Six months later, the retina was reattached; the VA improved to 0.1 in the left eye at the final examination (Fig 4F).

Discussion

Microincision vitrectomy surgery combined with preoperative IVB can be beneficial for treating TRD secondary to severe PDR. The therapeutic efficacy is based on 2 surgical principles: eliminating intraoperative complications by pharmacologic involution of retinal neovascularization and simplifying the surgical technique with fewer instrument exchanges. However, the effect of bevacizumab, that is, less intraoperative bleeding, and the efficacy of small-gauge instrumentation are difficult to evaluate objectively, and the synergistic impact of combining the 2 has not been studied.

In the current study, the baseline patient characteristics in the 2 groups did not differ significantly. Although the study eyes were recruited from 2 different study periods because of evolving innovations and refinements in the surgical system, the surgeries were conducted in consecutive patients in both groups. Therefore, the bias of case selection with different surgical procedures was eliminated. The primary retinal reattachment rates (95% vs. 91%) and ultimate reattachment rates evaluated at the final examination visit (100% in both group) did not differ between the groups, indicating that both techniques achieve favorable anatomic success in diabetic TRD.

The differences in the surgical instruments used for fibrovascular membrane removal and the methodology for achieving hemostasis during surgery between the 2 groups were compared, and the results demonstrated that the chances of using vitreoretinal forceps and membrane scissors during the removal of the membrane in the MIVS group was significantly lower than in the 20-g PPV group

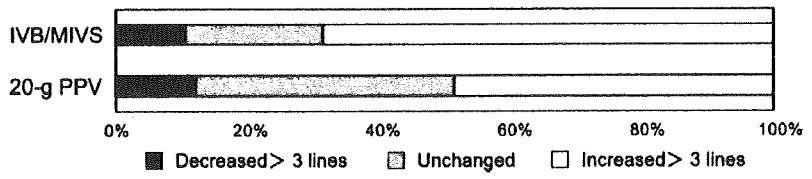

Figure 2. Bar graph showing the changes in the visual acuity at the 6-month postoperative follow-up examination in the 2 study groups. The visual changes are defined as changes of 0.3 or more in logarithm of the minimum angle of resolution units. IVB/MIVS = intravitreal bevacizumab-assisted microincision vitrectomy surgery group; 20-g PPV = 20-gauge pars plana vitrectomy group.

Table 3. Intraoperative and Postoperative Complications after the Initial Surgery

	Intravitreal Bevacizumab/ Microincision Vitrectomy Surgery Group (n = 38)	20-Gauge Pars Plana Vitrectomy Group (n = 33)	P Value
Intraoperative complications			
Iatrogenic retinal breaks, no. (%)*	8 (21)	14 (42)	0.09
Mean scores of intraoperative bleeding†	2.7±0.9	3.7±0.6	<0.001
Postoperative complications, no. (%)			
Fibrin formation	9 (24)	15 (45)	0.09
Hyphema	3 (8)	1 (3)	0.62
Hypotony‡	1 (3)	0 (0)	1.00
Hypotony-induced maculopathy	1 (3)	0 (0)	1.00
Progressing or persistent NVG	3 (8)	2 (6)	1.00
Progressive fibrovascular proliferation	2 (5)	3 (9)	0.66
Persistent or recurrent VH	5 (13)	9 (27)	0.23
Recurrent retinal detachment¶	2 (5)	4 (12)	0.41
Vitreoretinal reoperation, no. (%)	4 (11)	8 (24)	0.22

IOP = intraocular pressure; NVG = neovascular glaucoma; VH = vitreous hemorrhage.
*Iatrogenic retinal breaks included the sclerotomy-related peripheral retinal breaks and retinal breaks encountered during posterior manipulations.
†The severity of intraoperative bleeding was graded based on the methodology for achieving hemostasis as follows: 0, self-limited bleeding without hemostatic procedures; 1, intraoperative hemostasis by gentle, direct depression of the bleeding point with a vitreous cutter; 2, hemostasis with elevated IOP; 3, hemostasis with both elevated IOP and direct cautery to the bleeding point.
‡Hypotony was defined as a postoperative IOP ≤7 mmHg.
¶Recurrent retinal detachment included traction retinal detachment and rhegmatogenous retinal detachment after the initial vitrectomy.

($P = 0.02$ and $P<0.001$, respectively). Because the 23- and 25-gauge vitrectomy ports are small and the distances from the ports to the tips are shorter than those of a conventional 20-gauge vitrectomy, the port of the small-gauge vitrectomy can be inserted readily between the fibrovascular membrane and retina, allowing successful membrane segmentation, dissection, and removal using a 23- or 25-gauge vitrectomy in most cases. In patients with combined TRD/RRD and extensively adhesive fibrovascular membranes, a bimanual technique consisting of use of a membrane forceps to grasp the membrane with 1 hand and a 23- or 25-gauge vitrectomy instead of scissors to separate the membrane from the retinal surface using the other hand can be performed easily. The membranes then can be removed using the vitrectomy with-

Table 4. Comparison of the Baseline Parameters in Eyes with or without Progression of Preexisting Traction Retinal Detachment after Intravitreal Bevacizumab

Biologic and Environmental Factors	Progression of Preexisting Traction Retinal Detachment		P Value
	Yes (n = 7)	*No (n = 31)*	
Mean age±SD, yrs	47.1±7.8	50.0±15.1	0.63
Preoperative parameters			
BCVA (logMAR)±SD	1.36±0.80	1.32±0.92	0.57
IOP (mmHg)±SD	16.1±5.7	18.4±9.9	0.42
Absence of previous PRP, no. (%)	4 (57)	4 (13)	0.025
Combined TRD/RRD, no. (%)	3 (43)	3 (10)	0.06
INV/NVG, no. (%)	0 (0)	5 (16)	0.56
Ring-shaped FVM formation, no. (%)	4 (57)	3 (10)	0.013
VH, no. (%)	2 (29)	15 (48)	0.39
Interval between IVB and MIVS (days)±SD	7.1±6.7	7.7±7.5	0.81
Systemic data			
HbA_{1c} (%)±SD	7.6±1.2	7.7±12.0	0.81
Creatinine (mg/dl)±SD	1.0±0.3	1.5±1.8	0.87

BCVA = best-corrected visual acuity; FVM = fibrovascular membranes; HbA_{1c} = hemoglobin A_{1c}; INV = iris neovascularization; IOP = intraocular pressure; IVB = intravitreal bevacizumab; logMAR = logarithm of minimal angle of resolution; MIVS = microincision vitrectomy surgery; NVG = neovascular glaucoma; PRP = panretinal photocoagulation; RRD = rhegmatogenous retinal detachment; SD = standard deviation; TRD = traction retinal detachment; VH = vitreous hemorrhage.

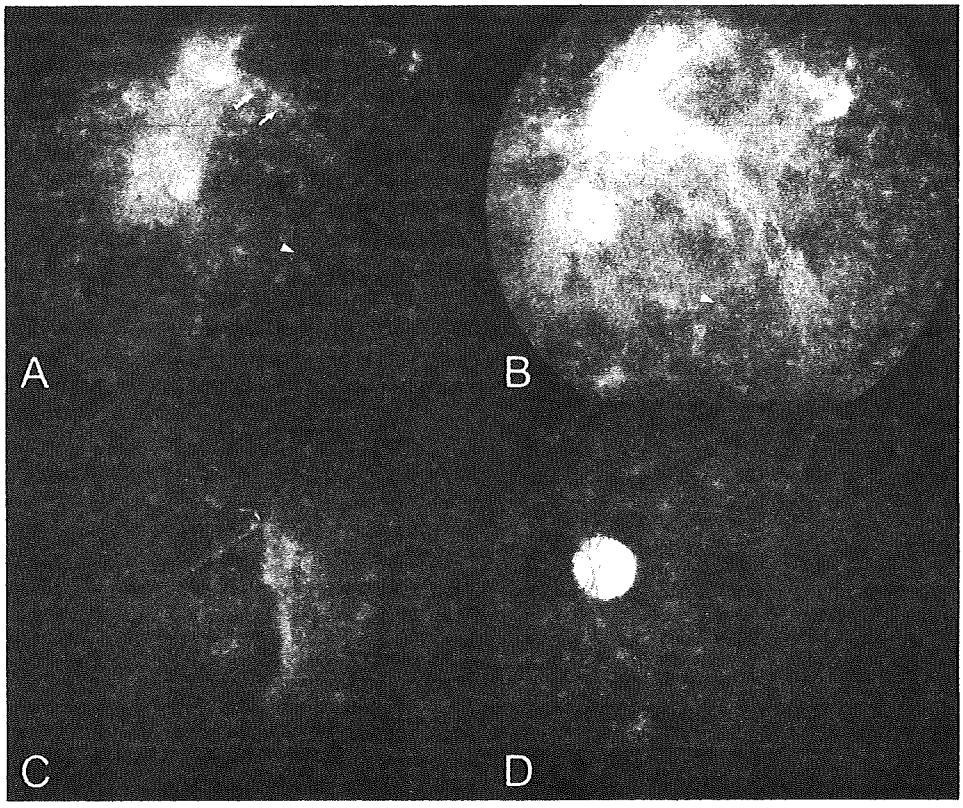

Figure 3. Case 1: a 46-year-old man with a tractional retinal detachment involving the macula in the left eye. **A**, Fundus photograph showing active fibrovascular membranes with traction involving the macula. Active retinal neovascular components (arrows and arrowhead) and subhyaloidal hemorrhages are noted. The visual acuity (VA) was 0.06. **B**, Fundus photography obtained 4 days after a 1.0-mg injection of intravitreal bevacizumab showing that the neovascular components regressed with a marked reduction in size of many neovascular vessels (arrows and arrowhead) and fibrotic advancement in the fibrovascular membrane. **C**, Intraoperative photograph obtained during 25-gauge microincision vitrectomy surgery. The fibrotic membrane can be segmented and dissected simply using a 25-gauge vitrectomy with only slight intraoperative bleeding. **D**, Fundus photography obtained 1 month after surgery showing that the retina has reattached successfully. The VA improved to 0.15 at the 6-month postoperative examination.

out instrument exchanges. No eyes required conversion to the conventional 20-gauge vitrectomy technique for membrane removal. Use of a blunt membrane forceps and a vitrectomy for membrane separation and dissection may be safer than using conventional sharp 20-gauge instruments to prevent iatrogenic retinal breaks. This may account for the lower, although not significant ($P = 0.11$), incidence (19%) of iatrogenic breaks during membrane removal in the IVB/MIVS group compared with 41% in the 20-gauge PPV group.

Intraoperative bleeding during removal of a fibrovascular membrane was observed in all cases whether conventional 20-gauge sharp instruments or small-gauge blunt instruments were used. However, achieving hemostasis in the IVB/MIVS group seemed much easier than in the 20-gauge PPV group, as reflected by the substantial differences in the scoring of the intraoperative bleeding between the groups. Of the 31 eyes in the IVB/MIVS group, the neovascular component of the fibrovascular membranes regressed with the reduced size of the abnormal new vessels during surgery. Most bleeding during membrane dissection or peeling was minor and some stopped spontaneously. Even if prominent bleeding occurred during membrane removal, it stopped in most cases with direct compression of the bleeding point with the blunt tip of the vitrectomy. This technique (see Video 3, available at http://aaojournal.org) is feasible using small-gauge instruments and is attributed partly to the fact that bevacizumab reduces the retinal neovascular activity. Reducing the use of cautery to achieve hemostasis may eliminate coagulative damage to the normal retinal vessels and time wasted in instrument exchanges.

The significantly shorter operating time in the IVB/MIVS group was noteworthy. Although a previous study[42] that compared the surgical times between 25- and 20-gauge vitrectomy reported that the 5 to 10 minutes needed for wound creation and closure in the 25-gauge group are equalized by the longer vitrectomy because intraoperative manipulation may cause more difficulties with a small-gauge system, the current results showed that the shortened time of approximately 40 minutes in the IVB/MIVS group not only was associated with easy wound creation and closure but also was more likely related to efficient intraoperative manipulation.

The incidence of postoperative complications in this study was similar between groups. As reported recently, postoperative development of anterior segment neovascularization, that is, INV/NVG, no longer was common in modern diabetic vitrectomy,[43] and no new INV/NVG was

encountered during follow-up. However, progression or recurrence of preexisting INV/NVG occurred in both groups. Furthermore, preoperative injection of bevacizumab also failed to prevent postoperative hemorrhages in all patients in the IVB/MIVS group. Bevacizumab may be washed out during vitrectomy and the severity of retinal ischemia may be underestimated or masked by bevacizumab-induced regression of neovascularization; hence, recurrence of neovascularization and vitreous hemorrhage may appear soon after surgery before the intraoperative endolaser treatment is effective. Because preoperative and postoperative INV/NVG and postoperative vitreous hemorrhage cause severe vision loss after surgery,[13] reinjection of bevacizumab should be considered at the end of surgery.

The incidence of postoperative retinal redetachment did not differ significantly between groups, that is, 5% in the IVB/MIVS group and 12% in the 20-gauge PPV group, and was comparable with rates reported recently (3% to approximately 11%).[25,43] The recent preference for a panoramic viewing system during vitrectomy and current surgeon recognition of not creating a sclerotomy has reduced the occurrence of sclerotomy-related breaks and related postoperative retinal redetachments. Instead, progressive fibrovascular proliferation is still a major postoperative complication that can cause subsequent retinal redetachment. Intravitreal injection of an anti-inflammatory, an anti-VEGF agent, or both, at the end of surgery may suppress intraocular reproliferation after surgery for severe PDR.

Cataract progression is a major vision-threatening complication after diabetic vitrectomy.[6,25] Because crystalline lens-sparing diabetic vitrectomy tends to have a higher subsequent reoperation rate,[43] phacoemulsification and IOL implantation were combined with vitrectomy in the presence of lens opacification.[19,44] As a result, there were fewer phakic patients after surgery in both groups in the current study who could be evaluated for postoperative cataract progression. At the same time, VA measurement 6 months after surgery can be affected less by postoperative cataract progression, and the postoperative VA seemed to reflect directly the impact of diabetic vitrectomy on visual prognosis in this series. The mean postoperative VA at the 6-month visit in the IVB/MIVS group (0.30) was better than that in the conventional 20-gauge PPV group (0.22) and reached borderline significance ($P = 0.047$), despite comparable baseline VAs in both groups. However, a strong impact of IVB/MIVS on visual recovery in treating diabetic TRD could not be shown because the mean VA changes at the 6-month evaluation did not differ significantly between the 2 groups ($P = 0.18$). Nevertheless, the higher percentage of eyes with more than 3 lines of visual improvement in the IVB/MIVS group (68%) may suggest that the shorter

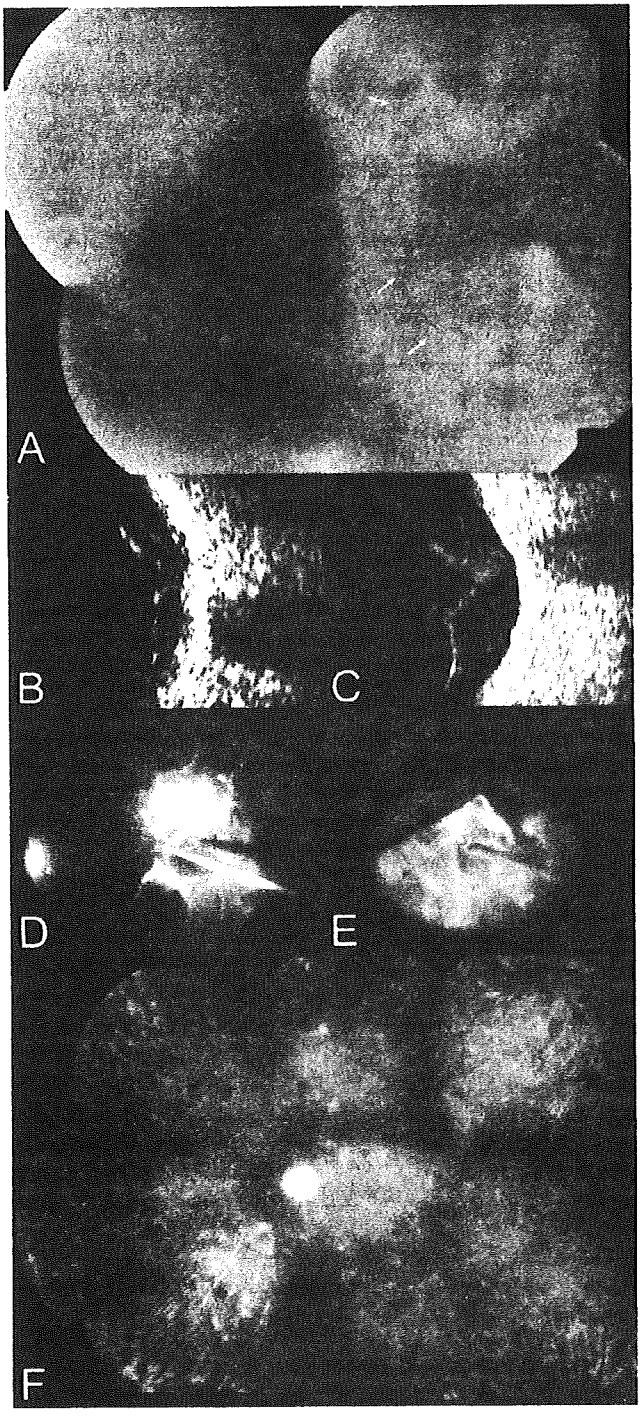

Figure 4. Case 2: a 48-year-old woman with a tractional retinal detachment (TDR) complicated by a dense cataract in the left eye. **A**, Fundus photograph showing, despite being obscured by lens opacification, extensive fibrovascular membranes with active neovascular components (arrows). **B**, B-scan echography showing a shallow but extensive TRD involving the macula. The visual acuity (VA) was 0.02. **C**, B-scan echography obtained 4 days after a 1.0-mg injection of intravitreal bevacizumab showing progression of the preexisting TRD with retinal elevation and a presumed subretinal hemorrhage. **D**, Intraoperative photograph obtained during primary 25-gauge microincision vitrectomy surgery (MIVS) showing a total retinal detachment with extensive contraction of the fibrotic membrane. **E**, Intraoperative photograph showing that the adherent fibrovascular membranes are separated and dissected bimanually using a 25-gauge membrane forceps and vitrectomy. **F**, Fundus photography obtained 6 months after surgery showing that the retina has reattached successfully after repeated MIVS. The VA improved to 0.1 at the final examination.

surgical time with fewer intraoperative complications seems to favor visual recovery synergistically using IVB/MIVS. However, a prospective, randomized study is needed to clarify this.

The theoretical concerns about MIVS are the increased risk of postoperative hypotony and endophthalmitis.[19,45,46] Although 1 case of postoperative hypotony-induced maculopathy occurred during the early study period in the IVB/MIVS group, postoperative hypotony no longer occurs because the surgical procedure was modified to a 2-step entry with aggressive suture placement in cases with leaky sclerotomies. The 74% self-sealing rate in the MIVS group was much lower than that reported previously[15,16,18] and may be attributed to extensive peripheral vitreous shaving. Instead, that procedure and endolaser photocoagulation to the anterior retina in this series may have contributed to the absence of fibrovascular ingrowth around the sclerotomy or anterior hyaloid fibrovascular proliferation. Endophthalmitis did not develop during follow-up. The overall incidence of vitreoretinal reoperation for treating postoperative complications in the MIVS group (11%) was much lower than that in the 20-gauge PPV group (24%). However, the small numbers of patients in both groups limited the ability to determine the effectiveness of IVB/MIVS for reducing the reoperation rate of diabetic vitrectomy.

Although systemic and local adverse events associated with IVB have been reported,[47,48] the rapid development or progression of TRDs induced by IVB is recognized as a new concern when treating severe PDR and retinopathy of prematurity.[49,50] In the current study, 7 eyes had acute progression of a TRD between adjunctive IVB and MIVS. The incidence of 18% in eyes with a preexisting TRD was much higher than in a recent report (5.2%) in which IVB was performed not only to treat diabetic TRD but also for other complications resulting from severe PDR.[49] Therefore, the authors postulated that IVB-induced progression of TRD may occur more easily in eyes with a preexisting retinal detachment. Although progression of TRD in active PDR may be the result of natural history, all cases reported here had extremely rapid contraction after IVB that was accompanied by marked involution of the neovascular components and extensive fibrotic changes in the fibrovascular membrane. Fibrotic progression and posterior hyaloid contraction in response to the acutely decreased VEGF level may increase the tractional force and may accelerate further progression of the preexisting retinal detachment. Progression of TRD involving the macula may cause serious vision loss even after successful anatomic retinal detachment repair. This possible complication should be recognized, and a timely surgical approach should be considered after IVB. Although the time between the injection and scheduled MIVS (mean, 7.6 days; range, 2–30 days) varied widely in the current study, a significant association was not found between the interval between IVB and the scheduled MIVS and progression of TRD. Instead, the ring-shaped fibrovascular membrane formation and absence of laser photocoagulation were newly explored as the risk factors for progression of TRD after IVB in eyes with a preexisting diabetic TRD in this series. Although the current results do not negate the possibility that a longer interval between IVB and surgery may affect the progression of TRD, caution should be taken in patients with diabetic TRD with these relevant findings. Bevacizumab overdosing may be another factor that contributes to the rapid progression of a TRD. A recent study reported that 1.25 mg bevacizumab can decrease high aqueous humor VEGF concentrations to under the physiologic level in patients with diabetic TRD.[51] A much lower dose of bevacizumab may hasten neovascular regression sufficiently.[32] Although numerous studies have reported the efficacy of bevacizumab for treating neovascular diseases, the appropriate dose for an intravitreal injection is unknown because of the off-label use of IVB. Further studies should determine the appropriate dose of IVB.

The current study had several limitations: its retrospective nature, the absence of randomization, and the different study periods of the 2 groups. The duration of retinal detachments involving the macular region, which could not be documented accurately, might have affected the visual outcomes. Minor differences in surgical techniques among the 4 surgeons also may have introduced bias into the anatomic results. Nevertheless, a consecutive series of eyes repaired with the same approach during each of the 2 different periods can eliminate the bias in case selection.

In conclusion, the results demonstrate favorable anatomic and 6-month postoperative VA results with minimal intraoperative complications and good surgical efficiency in MIVS assisted by IVB as a preoperative adjunct for treating diabetic TRD. However, patients should be followed up closely after IVB and should undergo a timely MIVS to avoid TRD progression, especially in the presence of a ring-shaped fibrovascular membrane, no previous laser treatment, or both. A large, prospective, controlled study is recommended to determine the appropriate dose of bevacizumab and the long-term safety of MIVS combined with IVB to treat TRD secondary to severe PDR.

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Pharmacokinetics of Bevacizumab after Topical, Subconjunctival, and Intravitreal Administration in Rabbits

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Purpose. To investigate the pharmacokinetics of bevacizumab in rabbits for three different routes of administrations: intravitreal injection, subconjunctival injection, and eye drops.

Methods. Pigmented rabbits received bevacizumab in one eye by topical eye drops (1.25 mg/0.05 mL six times daily for the first 7 days), single subconjunctival injection (1.25 mg/0.05 mL), or single intravitreal injection (1.25 mg/0.05 mL). Bevacizumab concentrations in plasma and ocular tissues in the treated and fellow eyes were determined by sandwich enzyme-linked immunosorbent assay at 1, 2, 4, and 12 weeks after administration.

Results. After intravitreal injection in the treated eye, the mean maximum concentrations (C_{max}) of bevacizumab in the iris/ciliary body and retina/choroid were 109,192.6, and 93,990.0 ng/g, respectively, whereas after subconjunctival injection, the C_{max} was 1418.7 and 295.8 ng/g, respectively. In the fellow eyes, when the drug was administered by intravitreal injection, the C_{max} was 753.6 ng/g in the iris/ciliary body and 224.2 ng/g in the retina/choroid and by subconjunctival injection was 1192.9 and 187.0 ng/g, respectively. With eye drops, only a small level of bevacizumab was detected in the iris/ciliary body and retina/choroid. Systemic exposure to bevacizumab was at the same level when administered by intravitreal or subconjunctival injection.

Conclusions. Intravitreal injection of bevacizumab was the most effective route of administration for intraocular tissue. Also, bevacizumab injected subconjunctivally was transported into the intraocular tissues of the treated eyes at an effective level. Both intravitreal and subconjunctival injections of bevacizumab resulted in high plasma concentrations. Bevacizumab was distributed into the intraocular tissues in fellow eyes via the systemic circulation. This treatment may be effective for blocking vascular endothelial growth factor activity. (*Invest Ophthalmol Vis Sci.* 2009;50: 4807-4813) DOI:10.1167/iovs.08-3148

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Bevacizumab is a full-length humanized monoclonal antibody that binds all isoforms of vascular endothelial growth factor (VEGF). Intravitreal injection of bevacizumab is effective and widely used in age-related macular degeneration to prevent the development of choroidal neovascularization,[1-3] to prevent retinal neovascularization in proliferative diabetic retinopathy,[4-6] and to treat macular edema in diabetic retinopathy,[7,8] retinal vein occlusion,[9,10] and uveitis.[11,12] In addition, intravitreal bevacizumab has been used to prevent iris neovascularization.[13,14]

The possible effects of intravitreal injection of bevacizumab on the noninjected fellow eye have been reported previously.[4,15-18] These effects may be due to the possibility that bevacizumab enters the fellow by the systemic circulation. In rabbit eyes, Bakri et al.[19] measured the pharmacokinetics of bevacizumab in the aqueous and vitreous humors in the administrative and the fellow eye as well as in the plasma after intravitreal injection and demonstrated that bevacizumab was detected in the aqueous and vitreous humors in the fellow eye and the plasma.

One area of investigation for this drug has been how well it penetrates and localizes to the retina, choroid, and iris/ciliary body after intravitreal injection. Three groups demonstrated previously that after intravitreal injection, bevacizumab penetrated the retina in the mouse eye,[20] rabbit eye,[21] and monkey eye.[22] Recently, Peters et al.[23] showed that after intravitreal injection into the primate eye, bevacizumab was located in the blood vessel walls of the iris and ciliary body. However, there are no studies in the current literature that have measured the concentration of bevacizumab in the retina/choroid and iris/ciliary body as target tissues for neovascularization.

Bevacizumab is commonly administered by intravitreal injection, but recently it has been shown to be effective when administered subconjunctivally[24-27] and topically (eye drops),[28-30] in corneal neovascularization as well as experimentally in rabbit corneal neovascularization models.[31,32] However, the pharmacokinetics of bevacizumab when administered subconjunctivally and topically has yet to be investigated until now.

In this study we investigated the pharmacokinetics of bevacizumab in the treated and fellow eyes, and in plasma in rabbits for three different routes of administration: intravitreal injection, subconjunctival injection, and eye drops. We also investigated the contribution of topical and systemic absorption in the retina/choroid and iris/ciliary body for three administration routes.

Material and Methods

Animals

Seventy-two Dutch Belted rabbits, weighing 1.9 to 2.5 kg each, were obtained from Biotek Co., Ltd. (Saga, Japan). The animals were treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. The animals were divided into three

TABLE 1. Comparison of Pharmacokinetic Parameters Administered by Eye Drops and Subconjunctival or Intravitreal Injection

Parameter/Route	Eye-Drops		SCJ		IVT	
	Treated	Fellow	Treated	Fellow	Treated	Fellow
$T_{1/2}$, wk						
Iris/ciliary body	NC	NC	1.80	NC	0.82	NC
Vitreous	NC	NC	2.29	NC	0.85	NC
Retina/choroid	NC	NC	2.85	NC	0.89	NC
Plasma	NC		1.75		1.85	
C_{max}, ng/g						
Iris/ciliary body	16.1	11.7	1418.7	1192.9	109192.6	753.6
Vitreous	1.7	0.4	11.1	9.3	59730.8	6.7
Retina/choroid	18.2	10.2	295.8	187.0	93990.0	224.2
Plasma	14.3		3733.1		2087.2	
$AUC_{0\text{-last}}$/D, (ng · wk/g)/mg						
Iris/ciliary body	1.8	1.2	1905.6	1094.1	125089.7	1640.8
Vitreous	0.2	0.1	44.7	33.6	68353.0	8.0
Retina/choroid	1.9	1.3	645.1	440.5	179438.5	750.5
Plasma	1.4		8945.7		6088.9	
Effective duration, wk						
Above 22 ng/mL						
Iris/ciliary body	NC	NC	8.4	NC	10.3	NC
Retina/choroid	NC	NC	8.6	5.2	11.7	8.0
Above 500 ng/mL						
Iris/ciliary body	NC	NC	0.3	NC	6.6	NC
Retina/choroid	NC	NC	NC	NC	7.7	NC

Pharmacokinetic parameters were calculated from mean values obtained at each time point. SCJ, subconjunctival injection; IVT, intravitreal injection; D, administered dose of bevacizumab; NC, not calculated.

groups: treatment by daily topical administration of bevacizumab (group 1), a single subconjunctival injection of bevacizumab (group 2), and a single intravitreal injection of bevacizumab (group 3).

Bevacizumab Administration

In group 1, 24 rabbits received 1.25 mg/0.05 mL of bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA) topically six times per day for the first 7 days in the right eye. In group 2, 24 rabbits received a single subconjunctival injection (1.25 mg/0.05 mL) of bevacizumab into the right eye, through a syringe (Hamilton, Reno, NV) with a 30-gauge needle, 3 to 4 mm from the limbus at the 12-o'clock position. In group 3, 24 rabbits received a single intravitreal injection (1.25 mg/0.05 mL) of bevacizumab into the right eye through the same size needle. Before an intravitreal or a subconjunctival injection, group 2 and 3 rabbits were systemically anesthetized with a mixture of xylazine hydrochloride (Celactal; Bayer Medical Ltd., Leverkeusen, Germany) and ketamine hydrochloride (Ketalar; Daiichi Sankyo Co., Ltd., Tokyo, Japan), and topically anesthetized with 0.4% oxybuprocaine hydrochloride (Benoxyl; Santen Pharmaceutical Co., Ltd., Osaka, Japan).

Enzyme-Linked Immunosorbent Assay for Bevacizumab

At predetermined intervals (1, 2, 4, or 12 weeks after administration), the rabbits were killed with an overdose of sodium pentobarbital (Somnopentyl; Kyoritsu Seiyaku Co., Tokyo, Japan), the eyes enucleated, and blood samples collected. The aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid were separated. The tissue samples of iris/ciliary body, vitreous, and retina/choroid were homogenized (CellLytic MT; C3228, Sigma-Aldrich, St. Louis, MO). Plasma was obtained from the blood sample for 10 minutes by centrifugation (600*g*) at room temperature. Bevacizumab concentrations in plasma, aqueous and vitreous humors, iris/ciliary body, and retina/choroid in the treated and the fellow eyes were determined by sandwich ELISA using 1 μg/mL of rabbit anti-human IgG (H+L) (AffiniPure, Catalog no: 309-005-082; Jackson ImmunoResearch, West Grove, PA) as a primary antibody and an ELISA kit (Protein Detector; Catalog no.: 54-62-10; Kirkegaard & Perry Laboratories, Inc., Gaithersburg, MD). The lower limit of quantification for this method was 0.1 ng/ mL.

Calculation of Pharmacokinetic Parameters

Pharmacokinetic parameters of drugs in the vitreous, iris/ciliary body, retina/choroid, and plasma including half-life ($T_{1/2}$), C_{max}, and the area under the curve $(AUC)_{0\text{-last}}$ were calculated from mean values obtained at each time point with commercial software (WinNonlin Professional, ver. 5.2; Pharsight Co. Mountain View, CA). $T_{1/2}$ was obtained to follow first-order kinetics. Relative contribution of topical and systemic absorption in the iris/ciliary body and retina/choroid of the treated eyes when administered by three different routes were calculated by the following equations:

$$\text{Contribution of topical absorption (\%)} = 100 \cdot [(C_{max(\text{treated eyes})} - C_{max(\text{fellow eyes})})/C_{max(\text{treated eyes})}] \quad (1)$$

$$\text{Contribution of systemic absorption (\%)} = 100 - (\text{contribution of topical absorption}) \quad (2)$$

RESULTS

Ocular Pharmacokinetics

Eye Drops. The concentration of bevacizumab in the aqueous humor, iris/ciliary body, vitreous humor, and retina/choroid after eye drop administration is shown in Table 1 and Figure 1. In the treated eyes (Fig. 1A), the C_{max} of bevacizumab in the iris/ciliary body and retina/choroid were 16.1 ± 2.8 and 18.2 ± 4.2 ng/g (mean ± SE) at 1 week after the start of drug administration, respectively. Topical administration was only on days 1 to 7. Two weeks after the start of topical administration, a very low level of bevacizumab was detected in the aqueous (0.6 ± 0.6 ng/ mL) and the vitreous (1.7 ± 0.3 ng/ mL) humors. In the fellow eyes (Fig. 1B), the C_{max} in the iris/ciliary body and retina/choroid were 11.7 ± 2.1 and 10.2 ± 1.2 ng/g, respectively, at 1 week after the