INTRAVITREAL BEVACIZUMAB (AVASTIN) FOR PERSISTENT NEW VESSELS IN DIABETIC RETINOPATHY (IBEPE STUDY)

1-Year Results

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Purpose: To evaluate the effect of intravitreal bevacizumab on area of fluorescein leakage from active new vessels (NVs) and on best-corrected visual acuity in patients with actively leaking NV associated with diabetic retinopathy unresponsive to panretinal photocoagulation.

Methods: A prospective open-label study of diabetic patients with actively leaking NV refractory to panretinal photocoagulation and best-corrected visual acuity worse than 20/40. Ophthalmic evaluation, including fluorescein angiography, was performed at baseline and at Weeks 1, 6, 12, 24, and 48 after intravitreal bevacizumab (1.5 mg/0.06 mL) injection. After Week 12, patients could receive additional intravitreal bevacizumab injections pro re nata, per the discretion of the treating ophthalmologist. Main outcome measures include change from baseline (at each study visit) in total area of fluorescein leakage from active NV and change from baseline in best-corrected visual acuity.

Results: Fifteen consecutive patients were included, and 12 completed the study. Mean ± SEM fluorescein leakage was 27.7 ± 6.2 mm² at baseline and was significantly lower at all visits post injection; at Week 6, no leakage was observed (P = 0.0001). The mean ± SEM logarithm of minimum angle of resolution best-corrected visual acuity improved from 0.90 ± 0.11 at baseline to 0.70 ± 0.12 at Week 48 (P = 0.0449). Throughout the 48-week study period, patients received a mean of 2.16 injections.

Conclusion: With 1-year follow-up, treatment with intravitreal bevacizumab was associated with reduced fluorescein leakage from persistent NV and improved visual acuity in patients with diabetic retinopathy unresponsive to panretinal photocoagulation.

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Retinal neovascularization represents an important risk factor for severe vision loss in patients with diabetes mellitus. About 60% of patients with proliferative diabetic retinopathy (PDR) respond to panretinal photocoagulation (PRP) with regression of neovascularization within 3 months. However, many patients require additional laser treatment, and 4.5% undergo pars plana vitrectomy despite PRP. Although severe central vision loss because of PDR can be prevented with PRP in most cases, this destructive, often painful, laser procedure is associated with decreased peripheral vision and an increased risk of macular edema.

Vascular endothelial growth factor (VEGF) has been implicated in human eye diseases characterized by neovascularization. Intravitreal injection of VEGF into normal primate eyes induces the same
pathologic processes seen in diabetic retinopathy, including microaneurysm formation and increased vascular permeability.\textsuperscript{9–11} Levels of VEGF in the vitreous are highly correlated with growth of new vessels (NVs).\textsuperscript{6–8} Blockage of VEGF has been associated with inhibition of iris neovascularization and suppression of retinal NV formation in primates.\textsuperscript{12,13} Taken together, these findings provide the rationale for anti-VEGF therapy in retinal vascular diseases associated with NV formation, such as diabetic retinopathy.

In pioneering work, Adamis et al\textsuperscript{14} reported the regression of retinal neovascularization after treatment of patients with diabetic macular edema with the anti-VEGF agent pegaptanib (Macugen). Subsequently, Avery\textsuperscript{15} reported the regression of retinal neovascularization in patients with PDR after intravitreal injection of bevacizumab. Since then, a rapidly increasing number of studies\textsuperscript{16–23} have shown that anti-VEGF agents may be helpful for the treatment of retinal neovascularization in PDR. On the basis of these promising reports, we conducted a prospective evaluation of the effect of intravitreal bevacizumab on area of fluorescein leakage (FLA) from active NVs and on best-corrected visual acuity (BCVA) in patients with PDR unresponsive to PRP.

Materials and Methods

The study protocol adhered to the tenets of the Declaration of Helsinki and was approved by the local institutional review board, and all participants gave written informed consent before entering into the study. All diabetic patients evaluated at the Retina and Vitreous Section of the Department of Ophthalmology, School of Medicine of Ribeirão Preto, and at UDAT—Hospedal de Olhos de Araquara, with a diagnosis of persistent neovascularization because of PDR unresponsive to PRP between September and November 2005 were invited to participate in the study. Throughout the study, measurement of BCVA was performed by a single certified examiner according to the Early Treatment Diabetic Retinopathy (ETDRS) protocol before any other study procedure. Ophthalmic evaluation was performed by a single retinal specialist (R.J.), and stereoscopic fundus photography and fluorescein angiography were performed by a single certified ophthalmic technician (D.C.). Study data were interpreted and analyzed by R. A. Costa, I. U. Scott, A. Messias, L. P. Cintra, and J. A. S. Ribeiro.

Patient Eligibility and Baseline Evaluation

Patients were included if they had 1) persistent neovascularization, defined as active NVs (fine retinal vessels with dilated buds or tips covered with hemorrhage or associated with recurrent vitreous hemorrhage or paucity of accompanying fibrous tissue and/or increased in extent compared with previous visit),\textsuperscript{24} unresponsive to complete PRP performed at least 4 months previously according to ETDRS guidelines\textsuperscript{25} (PRP was performed in 2 sessions composed of six hundred to eight hundred 500-μm spots per session) and 2) logarithm of minimum angle of resolution ETDRS BCVA of 0.3 (Snellen equivalent, 20/40) or worse. Exclusion criteria included 1) history of vitrectomy in the study eye; 2) tractional retinal detachment; 3) history of thromboembolic event (including myocardial infarction or cerebral vascular accident); 4) major surgery within the previous 6 months or planned within the next 28 days; 5) uncontrolled hypertension (according to guidelines of the seventh report of the Joint National Committee on Prevention, Detection, Evaluation, and Treatment of High Blood Pressure [JNC-7]),\textsuperscript{26} or 6) known coagulation abnormalities or current use of anticoagulative medication other than aspirin.

During the study enrollment period, persistent neovascularization was identified in 1 eye of 3 patients and in both eyes of 13 patients based on clinical examination and confirmed by fluorescein angiography. If both eyes were eligible for treatment, then the eye with worse visual acuity was included in the study. Fifteen eyes of 15 patients were included in the study (1 patient declined study participation because of fear of the intravitreal injection procedure).

At baseline, each patient had a detailed ophthalmologic examination including measurement of BCVA according to a standardized refraction protocol using a retroilluminated Lighthouse for the Blind distance visual acuity test chart (using modified ETDRS charts 1, 2, and R), as well as applanation tonometry, undilated and dilated slit-lamp biomicroscopic examinations (including lenticular status using the Lens Opacities Classification System III),\textsuperscript{27} and indirect funduscopic examination. Stereoscopic digital color fundus photography (UVi-60/EyeQ Pro; Canon, Tokyo, Japan) and fluorescein angiography were also performed (TRC-50IA/IMAGEnet; Topcon, Tokyo, Japan).

Treatment Assignment

At baseline, all patients received 1 intravitreal injection of 1.5 mg (0.06 mL) bevacizumab according to the intravitreal injection technique described elsewhere.\textsuperscript{28} After the injection, central retinal artery perfusion was confirmed with indirect ophthalmoscopy. Patients were instructed to instill 1 drop of 0.3% ciprofloxacin into the injected eye 4 times daily for 1 week after the procedure.
Follow-up Examinations, Retreatment, and Outcome Measures

Patients were scheduled for follow-up examinations at Weeks 1, 6, 12 (±1), 24 (±2), and 48 (±2) after treatment. The same procedures performed at baseline were performed at each study visit (e.g., ETDRS BCVA, complete ophthalmic examination, photography, and fluorescein angiography). Between Weeks 12 and 48, in addition to the study visits described above, patients received a comprehensive ophthalmic evaluation at 4-week intervals and patients could receive additional fluorescein angiography studies and/or intravitreal bevacizumab injections pro re nata, based on physician judgment. Systemic and local adverse events were monitored throughout the study, including changes in intraocular pressure (IOP) and lens status.

Two measures were used to evaluate the effects of bevacizumab: 1) change from baseline in the total FLA (measured in square millimeters) from active NVs (new vessels at the disk and/or new vessels elsewhere) (FLA)29 and 2) change from baseline in logarithm of minimum angle of resolution ETDRS BCVA. When there was more than 1 site of active NVs, all sites were included for analysis (Figure 1).

Statistical Analysis

One-way analysis of variance for repeated measures with Tukey multiple comparisons posttest was used to evaluate change from baseline in FLA, logarithm of minimum angle of resolution BCVA, IOP, and lens opacity severity (Lens Opacities Classification System III grade). The significance level adopted was 0.05.

Results

Twelve of the 15 patients completed the 48-week study treatment protocol. Patient 12 was lost to follow-up after Week 12. Patient 07 developed angina at Week 35 and further treatment with bevacizumab was withheld. Patient 10 had vitreous hemorrhage at Week 46, and data from his 48-week visit were not analyzed. Of the 15 participants, 8 (53%) were men and 7 (47%) were women. The mean ± SD age was 55.8 ± 12 years (median 57; range, 49–73 years). The mean ± SD duration of diabetes was 11.7 ± 4.4 years (median 12; range, 3–18 years). Twelve of the 15 patients (80%) had a glycosylated hemoglobin level above 7%. Baseline characteristics are summarized in Table 1.

No serious drug-related adverse events were observed for the 15 patients treated in this study aside from the patient who had angina at Week 35. Overall, the treatment procedure was well tolerated, and no clinical evidence of uveitis, endophthalmitis, or ocular toxicity was observed. The mean (±SEM) IOP at baseline was 14.9 ± 0.7 mm Hg, and there was no significant IOP elevation noted throughout the study period in any of the patients. Furthermore, no changes in lens
| Patient | Gender | Age, years | DM Type | DM Duration | HbA1C, % | NI | BCVA 0 | BCVA 1 | BCVA 6 | BCVA 12 | BCVA 24 | BCVA 48 | NV 0 | NV 1 | NV 6 | NV 12 | NV 24 | NV 48 |
|---------|--------|------------|---------|-------------|----------|----|--------|--------|--------|--------|--------|--------|-------|-----|-----|-----|-----|-----|-----|
| 1       | Male   | 52         | II      | 12          | 9.6      | 3   | 0.52   | 0.52   | 0.4    | 0.3    | 0.6    | 0.46   | 24.16 | 3.3  | 0   | 4.55 | 8.53 | 0.6 |
| 2       | Male   | 60         | II      | 16          | 11       | 2   | 0.3    | 0.24   | 0.3    | 0.24   | 0.06   | 0.3    | 14.15 | 0    | 0   | 8.55 | 9.87 | 12.34 |
| 3       | Male   | 39         | II      | 6           | 9.1      | 2   | 0.8    | 0.56   | 0.56   | 0.54   | 0.7    | 1.07   | 0     | 0    | 0.5 | 4.89 | 12.34 | 0   |
| 4       | Male   | 58         | II      | 14          | 9.4      | 2   | 1.14   | 1.1    | 1.1    | 1.1    | 1.06   | 10.38  | 0     | 1.8  | 2.73 | 0   |     |
| 5       | Male   | 50         | II      | 5           | 11       | 1   | 0.3    | 0      | 0      | 0      | 0.02   | 0.02   | 74.2  | 8.85 | 0   | 15.54 | 19.34 | 12.26 |
| 6       | Male   | 67         | II      | 14          | 9.3      | 2   | 0.96   | 0.86   | 0.84   | 0.94   | 0.92   | 0.9    | 38.53 | 2.97 | 7.45 | 2.54 | 2.07 |     |
| 7       | Female | 73         | II      | 10          | 7.1      | 2   | 1.6    | 1.64   | 1.6    | 1.6    | 1.32   | 10.91  | 1.89  | 0.63 | 8.53 |     |     |
| 8       | Female | 34         | I       | 15          | 6.5      | 3   | 0.42   | 0.42   | 0.42   | 0.42   | 0.4    | 0.4    | 89.16 | 33.43 | 0   | 15.6 | 0    | 0   | 0.2 |
| 9       | Female | 65         | II      | 12          | 6.7      | 2   | 1.24   | 1.1    | 1.3    | 1.3    | 1.24   | 1.24   | 17.38 | 1.07 | 3.48 | 30.75 | 6.1  |     |
| 10      | Female | 65         | II      | 18          | 7.8      | 2   | 0.96   | 0.7    | 0.6    | 0.64   | 0.7    | 0.7    | 25.07 | 3.99 | 5.3 | 39.82 |     |     |
| 11      | Female | 35         | I       | 16          | 6.3      | 3   | 0.3    | 0.3    | 0.34   | 0.32   | 0.3    | 0.3    | 34.75 | 9.59 | 6.55 | 0.48 | 1.4 |     |
| 12      | Male   | 56         | II      | 15          | 8.1      | 1   | 1.06   | 1.1    | 0.96   | 0.98   | 1      | 6.47   | 0     | 0    | 0   |     |     |
| 13      | Male   | 53         | II      | 10          | 10.1     | 2   | 1.1    | 0.84   | 0.84   | 0.84   | 0.7    | 0.78   | 31.59 | 2.61 | 1.56 | 1.68 | 0.66 |     |
| 14      | Male   | 62         | II      | 10          | 8.9      | 2   | 1.6    | 1.3    | 1.32   | 1.34   | 0.88   | 0.66   | 20.15 | 4.25 | 5.4 | 1.04 | 0   |     |
| 15      | Female | 68         | II      | 3           | 9.9      | 2   | 1.22   | 1.06   | 1.12   | 1.1    | 1.6    | 1.6    | 18.91 | 9.59 | 4.7 | 9.4 | 1.8 |     |
| Mean    | —      | 55.8       | —       | 11.7        | 8.7      | 2   | 0.90   | 0.77   | 0.77   | 0.78   | 0.78   | 0.70   | 27.79 | 5.43 | 5.50 | 9.7 | 3.12 |     |
| SD      | —      | 12.1       | —       | 4.4         | 1.5      | 0.6 | 0.44   | 0.47   | 0.46   | 0.47   | 0.43   | 0.45   | 24.36 | 8.46 | 4.79 | 12.04 | 4.60 |     |

DM, diabetes mellitus; HbA1C, glycosylated hemoglobin A1C; NI, number of injections; NV, total FlA from active NVs.
status were observed in any of the 12 eyes during the 48-week follow-up period. Minor local adverse events related to the treatment procedure, such as subconjunctival hemorrhage and foreign body sensation, were reported in 26.6% (4 of 15) and 6.6% (1 of 15) of patients, respectively. These events were transient and resolved in all patients by 1 week after injection.

**Outcome Measures**

The mean (±SEM) area of active NVs noted at baseline (27.7 ± 6.2 mm²) decreased significantly to 5.4 ± 2.1 mm² (P < 0.05) 1 week after treatment. At Week 6, absence of FLA from NVs was documented in all 15 eyes. Twelve weeks after injection, some FLA from areas of NV noted at baseline was observed in 14 of 15 eyes (93%); however, the mean ± SEM NV area of 5.5 ± 1.2 mm² was significantly smaller than at baseline (P < 0.05). At Weeks 24 and 48, NV area was still significantly reduced: 9.9 ± 3.2 mm² (P = 0.0083) and 3.1 ± 1.3 mm² (P = 0.0007), respectively (Table 1; Figures 2 and 3).

Among the 14 patients who completed the 24-week visit, 7 (50%) received a second intravitreal injection of bevacizumab between Weeks 12 and 24. Between Weeks 24 and 48, 6 of the 12 patients (50%) received a second intravitreal injection and 3 out of these 6 patients received a third intravitreal injection (Table 1).

The mean ± SEM logarithm of minimum angle of resolution ETDRS BCVA at baseline was 0.90 (20/160) ± 0.11 and improved significantly to 0.76 (20/125+2) ± 0.12 (P = 0.0010), 0.77 (20/125+2) ± 0.11 (P = 0.0020), 0.77 (20/125+2) ± 0.12 (P = 0.0018), 0.74 (20/100–2) ± 0.13 (P = 0.0249), and 0.70 (20/100) ± 0.12 (P = 0.0449) at Weeks 1, 6, 12, 24, and 48 weeks, respectively (Table 1; Figure 2).

**Discussion**

The present study demonstrates a strong temporal correlation between intravitreal injection of bevacizumab and a dramatic reduction of the FLA because of
active NVs; at 6 weeks after injection, all patients showed absence of FLA from NVs. Jorge et al.\(^{22}\) reported absence of FLA from NVs in 21 of 22 patients with persistent neovascularization because of PDR refractory to PRP after treatment with intravitreal ranibizumab; in the latter study, the maximum effect with ranibizumab was observed 1 week after injection.

Figures 2 and 3 demonstrate the change in FLA after treatment with 0.5 mg of intravitreal bevacizumab in

**Fig. 3.** Red-free, early, and late-phase fluorescein angiograms from a diabetic patient with persistent actively leaking NVs 7 months after panretinal laser photocoagulation (Case 2). Actively leaking NVs were present at baseline. At Weeks 1 and 6 after an intravitreal injection of 1.5 mg of bevacizumab, no FLA is present from NVs noted at baseline. Twelve weeks after injection, FLA recurred from NVs noted at baseline; however, area of leakage corresponds to 60% of that noted at baseline. At Week 24, FLA from NVs increased and another intravitreal injection of 1.5 mg of bevacizumab was administered, resulting in no FLA from NVs at Week 48.
the current study. The mean reduction in NV area compared with baseline was 80% at Week 1, 100% at Week 6, and 80% at Week 12.

Using FLA as a parameter for the assessment of anti-VEGF effect, results of the current study, in which intravitreal bevacizumab was the anti-VEGF drug investigated, may be compared with a previously published prospective study among a similar patient population, in which intravitreal ranibizumab was the anti-VEGF drug investigated. Similar methodology was used in both studies, which were conducted by the same investigative group. Comparatively, the effect of bevacizumab appears to last longer than that of ranibizumab, because by 12 weeks, there was an 80% reduction of the area of persistent neovascularization treated with bevacizumab versus 50% after treatment with ranibizumab. It has been speculated that the longer lasting effect of bevacizumab is because of its twice longer half-life in the vitreous cavity compared with ranibizumab, but a difference in the group of patients included in each study may also explain this difference, particularly because the CATT showed favorable results regarding the number of intravitreal injections of ranibizumab needed to control neovascular age-related macular degeneration, when compared with bevacizumab; perhaps affinity to VEGF and molecule size are other important characteristics to be considered regarding the effects of different anti-VEGF agents on neovascularization regression.

Although the patients in the current study had all received complete PRP before study enrollment and received intravitreal bevacizumab treatment during the study, none of them demonstrated full retinal neovascularization regression at Week 48. Other studies among treatment-naive patients with PDR who were treated with PRP with or without intravitreal anti-VEGF therapy also did not demonstrate full retinal neovascularization regression as expected per the results of the ETDRS, in which 60% of patients with PDR responded to PRP with regression of retinal neovascularization within a period of 3 months. In the current study, we included patients who responded poorly to PRP treatment, likely attributable because of their poor glycemic control because only 3 of them had an HbA1c below 7%. Indeed, Emmanuele et al reported more aggressive behavior of diabetic retinopathy in black patients and in white patients of Hispanic origin compared with non-Hispanic white patients, with glycemic control being the main factor responsible for this difference.

A limitation of the current study is the lack of standardized criteria for retreatment after Week 12; the decision to retreat was at the discretion of the treating ophthalmologist. Only 6 of 14 patients were retreated between the 12th and 24th week and 6 of 12 between the 24th and 48th week even though a larger number of patients showed active NV on fluorescein angiography. This limitation in study methodology was corrected in the more recent study using ranibizumab for persistent NV, and all patients with active NV were retreated every 3 months. By adopting this criterion, all 16 patients who completed the 48-week study received reinjection every 3 months.

In the present study, a significant improvement in BCVA compared with baseline was observed at all subsequent study visits. In one patient, some improvement early in the study period was because of partial reabsorption of vitreous hemorrhage. In the other patients, we believe that the BCVA improvement is attributable to a reduction in macular edema. In fact, a reduction in FLA was observed in the macular region of all patients who completed the 48 weeks of the study. The lack of optical coherence tomography is a limitation of the present study because it did not permit us to quantitatively assess improvement in macular edema observed on angiography. When this study was conceived at the end of 2005, our institution was not yet equipped with an optical coherence tomography machine. This deficiency was corrected in a more recent study, in which ranibizumab was used for persistent retinal neovascularization associated with PDR, and optical coherence tomography monitoring demonstrated a significant reduction in macular thickness compared with baseline at 1, 6, 24, and 36 weeks after administration of intravitreal ranibizumab. This reduction in macular thickness is consistent with recent results of large multicenter studies among patients with diabetic retinopathy.

The results of the current study are consistent with data from other investigations that showed the absence of any apparent association between intravitreal injection of bevacizumab and increased IOP, development or progression of cataracts, or increased rates of endophthalmitis or uveitis.

Intravitreal bevacizumab treatment was associated with rapid regression of persistent neovascularization and improvement in BCVA in patients with PDR refractory to PRP. There was one case of vitreous hemorrhage and no other sight-threatening complications of PDR, such as tractional retinal detachment. Larger studies are warranted to confirm the role of anti-VEGF therapy in the management of patients with PDR.

Key words: angiogenesis, diabetes, photocoagulation, bevacizumab, retinopathy, VEGF.
References


