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CLINICAL STUDY

Intravitreal bevacizumab (avastin) for proliferative diabetic retinopathy: 6-months follow-up

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Abstract

Aims **To study the effects of intravitreal bevacizumab (Avastin) on retinal neovascularization (RN) in patients with proliferative diabetic retinopathy (PDR).**
Methods **Retrospective study of patients with RN due to PDR who were treated with at least one intravitreal injection of 1.25 or 2.5 mg of bevacizumab. Patients underwent ETDRS best-corrected visual acuity (BCVA) testing, ophthalmoscopic examination, optical coherence tomography (OCT), and fluorescein angiography (FA) at baseline and follow-up visits.**
Results **Forty-four eyes of 33 patients with PDR and a mean age of 57.2-years (range: 23–82 years) participated in the study. Thirty-three eyes (75%) had previous panretinal photocoagulation (PRP). Twenty-seven eyes (61.4%) showed total regression of RN on fundus examination with absence of fluorescein leakage, 15 eyes (34.1%) demonstrated partial regression of RN on fundus examination and FA. Follow-up had a mean of 28.4 weeks (range from 24 to 40 weeks). BCVA and OCT demonstrated improvement ($P<0.0001$). Three eyes without previous PRP ('naive' eyes) and with vitreous haemorrhage have avoided vitreo-retinal surgery. One eye (2.2%) had PDR progression to tractional retinal detachment requiring vitrectomy, and one eye (2.2%) had vitreous haemorrhage with increased intraocular pressure (ghost cell glaucoma). No systemic adverse events were observed.**
Conclusions **Intravitreal bevacizumab resulted in marked regression of RN in patients with PDR and previous PRP, and rapid resolution of vitreous haemorrhage in three naive eyes. Six-months results of intravitreal bevacizumab at doses of 1.25 or 2.5 mg in patients with PDR do not reveal any safety concerns.**
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Keywords: avastin; bevacizumab; diabetic retinopathy; intravitreal injections; proliferative; retinal neovascularization

Introduction

Diabetic retinopathy remains a major threat to sight in the working age population in the developed world. Furthermore, it is increasing as a major cause of blindness in other parts of the world especially in developing countries.[1] Proliferative diabetic retinopathy (PDR) is a major cause of visual loss in diabetic patients. In PDR, the growth of new vessels from the retina or optic nerve, is thought to occur as a result of vascular endothelial grow factor (VEGF) release into the vitreous cavity as a response to ischaemia.[2–4] Because VEGF has been shown to play a major role in retinal neovascularization (RN),[2,3] although other factors may be involved as well,[5,6] anti-VEGF treatments have been hypothesized as an alternative adjunctive treatment for RN.[7,8]

Bevacizumab (Avastin™ Genentech Inc., San Francisco, CA, USA) is a complete full-length humanized antibody that binds to all subtypes of VEGF and is successfully used in tumour therapy as a systemic drug.[9] Recent studies have demonstrated the usefulness of

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an intravitreal injection of bevacizumab in the reduction of vascular permeability and fibrovascular proliferation in macular oedema secondary to central vein occlusion, RN secondary to PDR, and choroidal neovascularization secondary to age macular degeneration.[8,10–14] The amount of human retinal penetration for a complete full-length anti-VEGF antibody is not known at present. However, full thickness retinal penetration of intravitreal bevacizumab was observed in an animal model.[15,16] Additionally, intravitreal bevacizumab does not appear to be toxic to the albino rabbit retina at a concentration up to 2.5 mg.[17]

Panretinal photocoagulation (PRP) currently is the principal therapy for PDR, unless the patient already has extensive vitreous haemorrhage, which would preclude the possibility of laser photocoagulation. Neovascularization on and around the optic disc (NVD) and vitreous haemorrhage were found to be more frequently associated with severe visual loss despite PRP in the Diabetic Retinopathy Study (DRS) and Early Treatment Diabetic Retinopathy Study (ETDRS).[18,19] Long intervals between PRP sessions and the variable amount of time required for a favourable response may increase the incidence of complications due to the progression of PDR.[18,20] In fact, a single episode of PRP or shorter intervals between PRP episodes, although desirable in severe PDR and when the patient must travel long distances for treatment, are often associated with acute visual disturbances due to exudative choroidal detachment, retinal detachment, and macular oedema.[21–24]

The purpose of this retrospective study was to evaluate the effectiveness of intravitreal bevacizumab on RN in patients with PDR as a base for future studies in which bevacizumab may be used as an adjuvant treatment to PRP for PDR.

Patients and methods

We conducted a retrospective study in 44 eyes of 33 patients with RN in patients with PDR, who were treated with off-label intravitreal bevacizumab between September 2005 and August 2006 at five institutions in Venezuela, Costa Rica, Brazil, Argentina, and Peru. Institutional Review Board/Ethics Committee approval and patients' informed consent were obtained for this study at all five institutions. The off-label use of the drug and its potential risks and benefits were discussed extensively with all patients. Eyes that were previously treated with scatter photocoagulation, had prior focal/grid laser photocoagulation, and previous intravitreal triamcinolone injection were included if any of those therapies had been performed at least 6 months before intravitreal bevacizumab. An injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) of bevacizumab was given according to the discretion of the treating physician.

Baseline data included age, sex, type, and duration of diabetes mellitus. Patients also underwent clinical examination including best-corrected visual acuity (BCVA) measurement with ETDRS chart, applanation tonometry, fundus examination, fluorescein angiography (FA), and optical coherence tomography (OCT). In patients with clinical significant macular oedema, baseline central retinal characteristics was observed by OCT (Stratus III OCT, Carl Zeiss, Dublin, CA, USA) using six diagonal slow 6-mm radial line scans, with software versions 3.0 and 4.0, through a dilated pupil by a retina specialist.

A 0.18-ml aliquot of commercially available bevacizumab was prepared for each patient and placed in a tuberculin syringe using aseptic techniques. Bevacizumab was stored for up to 3 weeks under refrigeration at 4°C under sterile conditions, and the syringes were capped with a needle. After the eye had been prepared in a standard manner using 5% povidone/iodine, an eyelid speculum was used to stabilize the eyelids, and the injection of 1.25 mg (0.05 ml) or 2.5 mg (0.1 ml) of bevacizumab was given 3.5–4 mm posterior to the limbus, through the infero-temporal pars plana with a 30-gauge needle under topical anaesthesia or subconjunctival lidocaine. After the injection, intraocular pressure and retinal artery perfusion were checked, and patients were instructed to administer topical antibiotics for 7 days.

Patients were examined at 1, 2 weeks, and 1 month after the first injection and monthly thereafter. One, three and six months after initial injection, patient evaluation was performed using ophthalmic examination with slit-lamp biomicroscopy, OCT, and FA. Patients were included in this consecutive series if there was a minimum of 6-months follow-up. The main outcome measure was the change in RN defined as the change in the area of vitreous leakage from NVD and new vessels elsewhere (NVE) in the late phase of FA. Patients received reinjections only if RN was not totally resolved on ophthalmic examination or FA. Data was analysed by a paired Student's *t*-test and a Fisher's exact test when appropriate.

Monitored systemic conditions included myocardial infarction, stroke, systemic hypertension, thromboembolic diseases, and death. Blood pressure was measured prior to bevacizumab injection and at 1 and 2 weeks following each injection. Other systemic conditions were assessed by a thorough review of systems. If the patients were unable to attend a particular visit, a telephone interview was conducted to assess for possible systemic complications, and a new appointment was scheduled. We certify that all applicable institutional

and governmental regulations concerning the ethical use of human volunteers were followed during this research.

Results

We reviewed the clinical records of 33 consecutive patients (44 eyes) with PDR injected with intravitreal bevacizumab between September 2005 and August 2006. Patients had a mean follow-up of 28.4 weeks (range from 24 to 40 weeks). Our patients had a mean age of 57.2 years (range from 23 to 82 years), and 51.5% were female (16 men and 17 women). Twenty-three diabetic patients (69.7%) were insulin-dependent. The mean duration of diabetes was 17 years (range from 1 to 30 years). Thirty-five eyes (79.5%) were treated with an intravitreal injection of 2.5 mg of bevacizumab, and nine eyes (20.5%) with 1.25 mg of bevacizumab. Of the 33 eyes (75%) that were previously treated with scatter photocoagulation (Figure 1), 19 had prior focal/grid laser photocoagulation (Figure 2), and two patients had a previous intravitreal triamcinolone injection (Table 1). Seventeen eyes had clinical significant macular oedema (CSME) at biomicroscopic non-contact fundus examination with a 66- or a 78-D lens.

The mean baseline BCVA was log MAR = 1.21 and the final mean BCVA was log MAR = 0.70 ($P < 0.0001$). Final BCVA analysis by subgroups demonstrated that 12 eyes (27.3%) remained stable, 29 eyes (65.9%) improved two or more ETDRS lines of BCVA, and three eyes (6.8%) decreased two or more ETDRS lines of BCVA. OCT results were available for all 18 patients with CSME, the mean central macular thickness was 487.4 μm (range from 284 to 1082 μm), and decreased to a mean of 260.6 μm (range from 178 to 475 μm) at the end of follow-up ($P < 0.0001$). Final BCVA analysis by subgroups of patients with CSME demonstrated that 14 eyes (82.4%) improved two or more ETDRS lines of BCVA (Table 2).

Twenty-seven eyes (61.4%) showed total regression of RN on fundus examination with absence of fluorescein leakage (Figures 1 and 2), 15 eyes (34.1%) demonstrated partial regression of RN on fundus examination and FA, and two eyes (4.5%) of two patients showed no regression of RN. The first of those two patients who did not respond was treated with 1.25 mg of bevacizumab and had PDR progression to tractional retinal detachment requiring vitrectomy resulting in a poor final visual acuity (VA) (counting fingers) due to ischaemic optic neuropathy. The second patient was treated with 2.5 mg of bevacizumab and developed vitreous haemorrhage with increased intraocular pressure (ghost cell glaucoma). In addition, these two patients had previous PRP (Table 3).

Twenty-one eyes (47.7%) needed a second injection due to recurrence of neovascularization at a mean of

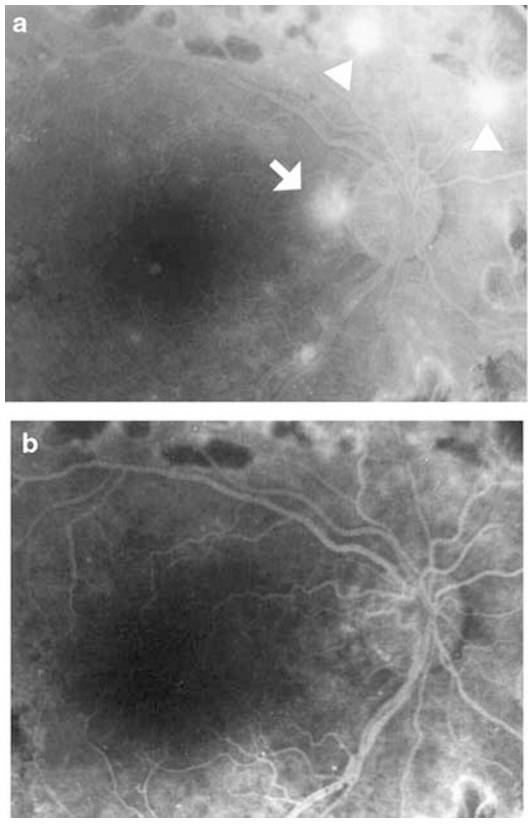

Figure 1 A 53-year-old man had a 2-month history of visual loss to 20/60 in his right eye. We had performed panretinal photocoagulation in his right eye 2 years previously. Fundus examination revealed a mild vitreous haemorrhage. (a) Fluorescein leakage from neovascularization of the disc (NVD) at baseline (arrow) between retinal vessels crossing the optic disc at 9 O'clock and 10 O'clock was demonstrated. In addition, FA showed magnification of retinal neovascularization elsewhere (NVE) in the superonasal retina (arrowheads). (b) At week 1 after intravitreal bevacizumab, total resolution of leakage from NVD and NVE are shown. His VA returned to 20/32 1 month later. He has not needed a reinjection at 5 months of follow-up.

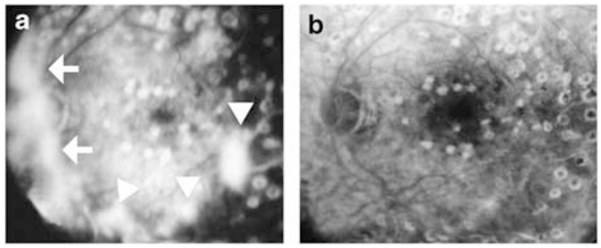

Figure 2 (a) Late-phase fluorescein angiogram demonstrating retinal neovascularization at the optic disc (NVD) (arrows) and neovascularization elsewhere (NVE) (arrowheads) in an 80-year-old man with proliferative diabetic retinopathy. He received a complete panretinal photocoagulation 2 years previously. (b) A fluorescein angiogram obtained 2 weeks after intravitreal bevacizumab injection demonstrated total regression of leakage from NVE and NVD in the late phase of the study.

12.4 weeks (range from 4 to 34 weeks), and seven eyes (15.9%) needed a third injection due to recurrence of neovascularization at a mean of 17.3 weeks (range from 11 to 22 weeks). Three eyes without previous PRP ('naive' eyes) and with vitreous haemorrhage have avoided vitreo-retinal surgery. There were no episodes of inflammation or severe decrease of vision immediately after an injection.

At 6 months, no systemic adverse events such as thromboembolic events (cerebrovascular accidents, transient ischaemic attacks, myocardial infarctions, or peripheral vascular disease) were reported.

Discussion

Although RN actually may be due to more than one cytokine, VEGF is an important, if not the most important cytokine involved.[25] Activation of the VEGF receptor pathway triggers a network of signalling processes that promotes endothelial cell growth, migration, survival from pre-existing vessels, differentiation, and mobilization of endothelial progenitor cells from the bone marrow into the peripheral circulation.[9,26,27] Furthermore, VEGF increases vessel permeability leading to deposition of proteins in the interstitium that facilitate the process of angiogenesis.[28] There are several reports published on the intravitreal administration of anti-VEGF compounds for RN in diabetic retinopathy.[7,13] In addition, there are five case reports on the use of intravitreal bevacizumab in RN in diabetic retinopathy demonstrating regression of RN in PDR.[14,29–32]

Our study demonstrated that intravitreal bevacizumab resulted in marked regression of RN on fundus examination and FA in patients with PDR and previous PRP. Furthermore, a rapid resolution of vitreous haemorrhage in three naive eyes was also seen. In addition, intravitreal bevacizumab demonstrated a similar beneficial response on macular thickness in eyes with PDR, and probably bevacizumab prevents exacerbation of macular oedema in patients with concomitant CSME and PDR. To determine the effect of an intravitreal injection of bevacizumab on actively growing new vessels, we chose the change in vitreous leakage from RN as our primary outcome. The detection of NVD and NVE on FA allowed the use of a systematic anatomical approach to monitor the area of leaking new vessels over time. Finally, to determine the effect of an intravitreal injection of bevacizumab on macular oedema, we measured the change of retinal thickening with OCT.

Regression of neovascularization and decrease of retinal thickening occurred in some injected eyes as soon as 7–15 days after the intravitreal injection of bevacizumab. Twenty-one eyes (47.7%) needed a second injection due to recurrence of neovascularization at a mean of 12.4 weeks, and seven eyes (15.9%) needed a

Table 1 Distribution of eyes according to prior treatment

Prior treatment	*2.5 mg IVT bevacizumab*	*1.25 mg IVT bevacizumab*	*Total of eyes*
PRP	12	2	14
PRP + grid	11	4	15
PRP + focal	2	2	4
Total of eyes	25	8	33

IVT, intravitreal; PRP, panretinal photocoagulation.

Table 2 Characteristics of patients with macular oedema associated to RN

Patient no.	*Prior treatment*	*Baseline VA log Mar*	*Baseline macular thickness by OCT (μm)*	*Final VA Log Mar*	*Final macular thickness by OCT (μm)*
1	PRP	0.3	284	0.1	244
4	No	1.0	1082	0.5	357
5	No	2.0	404	1.3	369
6	No	2.0	267	0.7	178
7	PRP + focal	1.3	559	1.2	178
9	PRP + grid	1.0	471	1.9	475
10	PRP	1.0	589	0.3	215
11	No	2.0	381	2.0	219
12	PRP + grid	1.8	615	0.2	209
13	PRP + grid	1.0	481	0.2	269
14	PRP + grid	1.0	481	0.3	263
15	PRP + grid	1.8	383	0.5	192
16	PRP + grid	1.3	355	0.5	213
17	PRP + grid	1.3	862	0.6	324
18	PRP + grid	1.0	362	0.5	203
19 (RE)	PRP	1.8	367	1.3	270
19 (LE)	No	2.0	343	1.3	252

CF, counting fingers; LE, left eye; OCT, optical coherence tomography; PRP, panretinal photocoagulation; RE, right eye; RN, retinal neovascularization; VA, visual acuity.

Table 3 Comparison between 2.5 and 1.25 mg of IVT bevacizumab for PDR

RN regression	*Neovascularization with previous PRP*		*Naive neovascularization*		*Total eyes*
	2.5 mg	*1.25 mg*	*2.5 mg*	*1.25 mg*	
Total	16	2	9	0	27
Partial	9	4	0	2	15
No	1	1	0	0	2
Total eyes	26	7	9	2	44

IVT, intravitreal; naive, no previous PRP; PDR, proliferative diabetic retinopathy; PRP, panretinal photocoagulation; RN, retinal neovascularization.

third injection due to recurrence of neovascularization at a mean of 17.3 weeks. Interestingly, we found that the 2.5 mg seems to be more effective than the 1.25 mg dose to induce complete regression of RN in naive eyes ($P = 0.01$; Table 3). The reason for this dose-dependant response on RN in naive eyes in unknown. In addition, the optimum dose and dosing sequence for intravitreal bevacizumab is still undetermined. We elected to defer reinjection only when there was a recurrence. Our clinical impression is that the effect of intravitreal bevacizumab on RN may be more lasting than in eyes with other pathologies such as choroidal neovascularization or macular oedema; however, the cause is not known.

Our results suggest an overall VA gain as well as a reduced risk of VA loss in eyes with diabetic macular oedema (as recognized on OCT) treated with intravitreal bevacizumab. We did not find any differences in the effectiveness between the doses of 1.25 and 2.5 mg for CSME, both of them demonstrated improvement with respect to VA and decrease in retinal thickness. Avery *et al*[13] reported similar results to the present study in 45 eyes of 32 patients with retinal and/or iris neovascularization secondary to diabetes mellitus who had received intravitreal injections of 6.2 μg–1.25 mg of bevacizumab. They demonstrated that all patients with neovascularization had complete or at least partial reduction in leakage of the neovascularization within 1 week after the injection. Additionally, they found in two cases, a subtle decrease in leakage of retinal or iris neovascularization in the fellow uninjected eye. We could not confirm their observation as in our study, utilizing higher doses (1.25–2.5 mg) of bevacizumab, all of our patients with bilateral RN underwent bilateral intravitreal injections.

Panretinal photocoagulation has been the mainstay for the treatment of PDR, and its suppressive effect on RN has been well documented.[20,21,33,34] However, substantial regression of new vessels may take weeks after completion of PRP, and in up to one-third of cases, new vessels continue to grow despite initial PRP.[21,34] In these cases, vitreous haemorrhage may induce visual loss and prevent complete laser. Moreover, macular oedema may increase after PRP and cause transient or persistent visual loss.[35,36] Our study demonstrates multiple benefits of intravitreal bevacizumab on PDR and in the future this new option could be an adjuvant agent to PRP so that more selective therapy may be applied. In addition, bevacizumab may allow long intervals between PRP sessions to avoid the development of macular oedema and other complications.[21–24]

The current study has several limitations, including a relatively small sample size and a relatively short duration of follow-up. In addition, this study included patients from five different centres and patients were treated according to the discretion of the treating physician. However, the large difference in the quantitative morphologic outcomes and the trend towards improvement in BCVA in injected eyes found at 6 months confirms our hypothesis that at least some eyes with PDR, such as those with pre-existing macular oedema or rapidly growing new vessels, may truly benefit from intravitreal bevacizumab. In addition, we can safely assume with a 95% confidence, that the true rate of systemic complications is $<9\%$ in our study.[37]

In summary, intravitreal bevacizumab seems to be a promising treatment for PDR, minimizing the risk for exudative complications, progression of RN, vitreous haemorrhage, and decreased vision caused by macular oedema. Intravitreal bevacizumab may potentially be used as an adjuvant agent to PRP for PDR. Although no serious complications of intravitreal injection of bevacizumab occurred in our series, further studies are needed to assess the efficacy and safety of intravitreal bevacizumab in the management of PDR.

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