European Journal of Ophthalmology / Vol. 18 no. 2, 2008 / pp. 263-269

Bevacizumab-augmented retinal laser photocoagulation in proliferative diabetic retinopathy: A randomized double-masked clinical trial

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PURPOSE. *To evaluate the additional therapeutic effect of single intravitreal bevacizumab injection on standard laser treatment in the management of proliferative diabetic retinopathy.*
METHODS. *A prospective, fellow-eye sham controlled clinical trial was conducted on 80 eyes of 40 high-risk characteristic proliferative diabetic retinopathy type II diabetics. All cases received standard laser treatment according to Early Treatment Diabetic Retinopathy Study protocol. Avastin-assigned eyes received 1.25 mg intravitreal bevacizumab (Genentech Inc., San Francisco, CA) on the first session of their laser treatments. Fluorescein angiography was performed at baseline and at weeks 6 and 16, and proliferative diabetic retinopathy regression was evaluated in a masked fashion.*
RESULTS. *The median age was 52 years (range: 39–68) and 30% of the participants were male. All patients were followed for 16 weeks. A total of 87.5% of Avastin-injected eyes and 25% of sham group showed complete regression at week 6 of follow-up ($p<0.005$). However, at week 16, PDR recurred in a sizable number of the Avastin-treated eyes, and the complete regression rate in the two groups became identical (25%; $p=1.000$); partial regression rates were 70% vs 65%. In the subgroup of Avastin-treated eyes, multivariate analysis identified hemoglobin A1c as the strongest predictor of proliferative diabetic retinopathy recurrence ($p=0.033$).*
CONCLUSIONS. *Intravitreal bevacizumab remarkably augmented the short-term response to scatter panretinal laser photocoagulation in high-risk characteristic proliferative diabetic retinopathy but the effect was short-lived, as many of the eyes showed rapid recurrence. Alternative dosing (multiple and/or periodic intravitreal Avastin injections) is recommended for further evaluation. (Eur J Ophthalmol 2008; 18: 263-9)*

KEY WORDS. *Proliferative diabetic retinopathy, Anti-VEGF bevacizumab, Hemoglobin A1c, Retinal laser photocoagulation*

Accepted: November 24, 2007

INTRODUCTION

Diabetic retinopathy remains the leading cause of blindness in the developed world for ages 20 through 74 years (1) and retinal neovascularization represents an important risk factor for severe visual loss in diabetic patients (2, 3).

The ischemic retina produces a new vessel-stimulating factor called vascular endothelial growth factor (VEGF) (2, 4, 5) whose ocular level is tightly correlated with both the growth and permeability of new vessels (2). Introduction of VEGF into normal primate eyes induces the same pathologic process seen in diabetic retinopathy (6, 7) and blockage of VEGF has been as-

 1120-6721/263-07$25.00/0

sociated with suppression of retinal new vessel formation (8, 9). These findings provide the rationale for anti-VEGF therapy in retinal vascular diseases associated with new vessel formation (10) such as diabetic retinopathy. Several clinical trials are currently evaluating the role of anti-VEGF agents for the treatment of ocular disease associated with choroidal and/or retinal neovascularization and exudative processes. Intravitreal injection of pegaptanib gained Food and Drug Administration approval for the treatment of neovascular age-related macular degeneration in 2004 (11), and it has shown encouraging results for the treatment of diabetic retinopathy (10, 12). Intravitreal Avastin (13-18) and Lucentis (19-21) have also been evaluated in several case series of proliferative diabetic retinopathy or diabetic macular edema and some beneficial effects were observed and further studies were recommended.

Severe central vision loss due to proliferative diabetic retinopathy (PDR) can be prevented by panretinal laser photocoagulation (PRP) in 50 to 60% of cases (22). In different studies based on a variety of defining criteria for regression of PDR and follow-up periods of 1, 3, or 6 months, retinal neovascularization showed regression in 38%, 59%, and 77% of patients (23-25). It is noteworthy that many of these cases may require additional laser treatment and 4.5% of them need pars plana vitrectomy (26).

The purpose of the current study was to evaluate the additional therapeutic effect of a single intravitreal injection of bevacizumab (Avastin; Genentech Inc., San Francisco, CA) on standard laser treatment in high-risk characteristic (HRC) PDR patients in terms of regression of retinal neovascularization. We also assessed the role of systemic factors in stability of PDR regression.

METHODS

Patients and examinations

The study was a fellow-eye controlled clinical trial. Eighty eyes of 40 bilateral HRC-PDR type II diabetics were included from December 2005 to September 2006. High-risk characteristics identified by Diabetic Retinopathy Study (DRS) criteria: neovascularization of the disc greater than or equal to one-forth to one-third disc area, any amount of disc neovascularization with vitreous or preretinal hemorrhage, or neovascularization elsewhere greater than or equal to one-half disc area with preretinal or vitreous hemorrhage (with or without macular edema). Fellow eyes of each case were randomly assigned to receive Avastin or sham. Patients with uncontrolled hypertension, recent (in the past 6 months) myocardial infarction or cerebrovascular accident, uncontrolled glaucoma, a history of any kind of retinal photocoagulation, or a diagnosis of tractional retinal detachment were excluded.

Comprehensive ocular examination with intraocular pressure measurement was performed at baseline. Blood pressure was monitored by the study observers. Hemoglobin A1c (HbA1c) and lipid profile were tested at baseline and at the last follow-up; averages were used in statistical analyses. Fluorescein angiography was performed at baseline and at weeks 6 and 16 post-intervention.

Intervention and follow-up

All cases received standard laser treatment (according to Early Treatment Diabetic Retinopathy Study protocol), i.e., PRP (1200–1500 spots, 200 ms duration, ½ spot size apart) and focal or grid macular photocoagulation for clinically significant macular edema (CSME).

Avastin-assigned eyes, which were selected randomly, received 1.25 mg (0.05 cc) bevacizumab (Genentech Inc.) on the first session of their laser treatment (following laser therapy). PRP was completed in three sessions, 1 week apart.

Bevacizumab is commercially available in 100-mg vials as a 25 mg/mL solution. Using an aseptic technique under a laminar flow hood, a compound pharmacist aliquoted 0.12 mL of the solution into multiple 1-mL polypropylene tuberculin syringes. The syringes were labeled and stored in a refrigerator at 6° C until used for injection; all were used within a week.

The injection was performed in the operating room and under topical anesthesia observing sterile conditions. First we did an anterior chamber paracentesis and then intravitreal injection was carried out through supratemporal pars plana. Fellow eye injection was mimicked with a needleless syringe. The patients were examined on the first day, at weeks 1, 2, 3, and 6, and then monthly thereafter; anterior chamber reac-

tion and/or intraocular pressure were specifically monitored and complications of cataract formation and vitreous hemorrhage were looked for. All patients were followed for at least 4 months; additional PRP was performed in case.

Statistical analysis

The main outcome measure of regression response was defined angiographically as follows: complete, defined as no leakage at minute 2 image; partial, defined as a decrease of leakage at minute 2 image in comparison with its baseline counterpart; and no response. This assessment was carried out by two independent masked observers; in case of conflict it was resolved through discussion. Fellow (Avastin- and sham-treated) eyes responses at week 6 and 16 follow-up were compared by McNemar test in a dichotomous fashion, i.e.; complete response vs partial or no response. The second main outcome measure—recurrence of PDR—was defined as deterioration from complete regression to partial/no regression status, or partial regression to no regression status from week 6 follow-up to week 16 follow-up.

Student *t* test was used to evaluate the association between the recurrence of PDR and age or HbA1c levels. Chi square test was used to assess the relationship of gender, hypertension, and hyperlipidemia with recurrence of PDR. When relevant, relative risks were calculated. Logistic regression was applied to control the interactions of the variables where the univariate analysis showed a significance lower than 0.2.

The study was conducted in conformance with the principles of Helsinki Declaration, and the institutional ethics committee approved the study protocol. Written informed consent was obtained from all participants before enrollment into the study.

Visual acuity data and optical coherence tomography findings of 26 cases with CSME (for which they underwent macular photocoagulation) are not presented as they were not directly related to the study aim.

RESULTS

The median age was 52 years (range: 39–68); 12 (30%) of the participants were male. Baseline mean HbA1c was 8.4%. Hypertension and hyperlipidemia were found in 42.5% and 47.5% of patients, respectively.

The treatment procedure was well tolerated; no clinical evidence of post injection uveitis, endophthalmitis, hemorrhage, and/or change in lens status was observed in follow-ups. No arterial embolic event was noted. Mean intraocular pressure readings at preoperative, week 1, 6, and 16 visits were comparable (Avastin vs sham and pre vs post-injection), and all were within the normal range (14.5–14.8 mmHg).

At week 6, 87.5% of the Avastin-injected eyes and 25% of the sham group showed complete regression on angiography ($p<0.005$). At week 16 this figure was 25% for both groups; 10 subjects had complete regression bilaterally, and no single eye in the remaining 30 participants maintained complete regression ($p=1.000$) (Tab. I). The recurrence of PDR at week 16 (following regression) is illustrated in Figure 1.

In the subgroup of Avastin-treated cases, recurrence of PDR in univariate analysis was associated with hypertension, female gender, and higher HbA1c levels, but in logistic regression analysis, only HbA1c maintained a significant relationship (Tab. II).

TABLE I - RESPONSE TO PANRETINAL PHOTOCOAGULATION IN AVASTIN AND SHAM GROUPS

Groups	Week 6 follow-up			Week 16 follow-up		
	Complete regression	Partial regression	None	Complete regression	Partial regression	None
Avastin (n=40)	35 (87.5)	5 (12.5)	0	10 (25)	28 (70)	2 (5)
Sham (n=40)	10 (25)	26 (65)	4 (10)	10 (25)	26 (65)	4 (10)

Values are n (%). McNemar p values were <0.005 and 1.000 for week 6 and 16 follow-ups, respectively; responses were compared dichotomously, i.e., complete regression vs partial regression or no response.

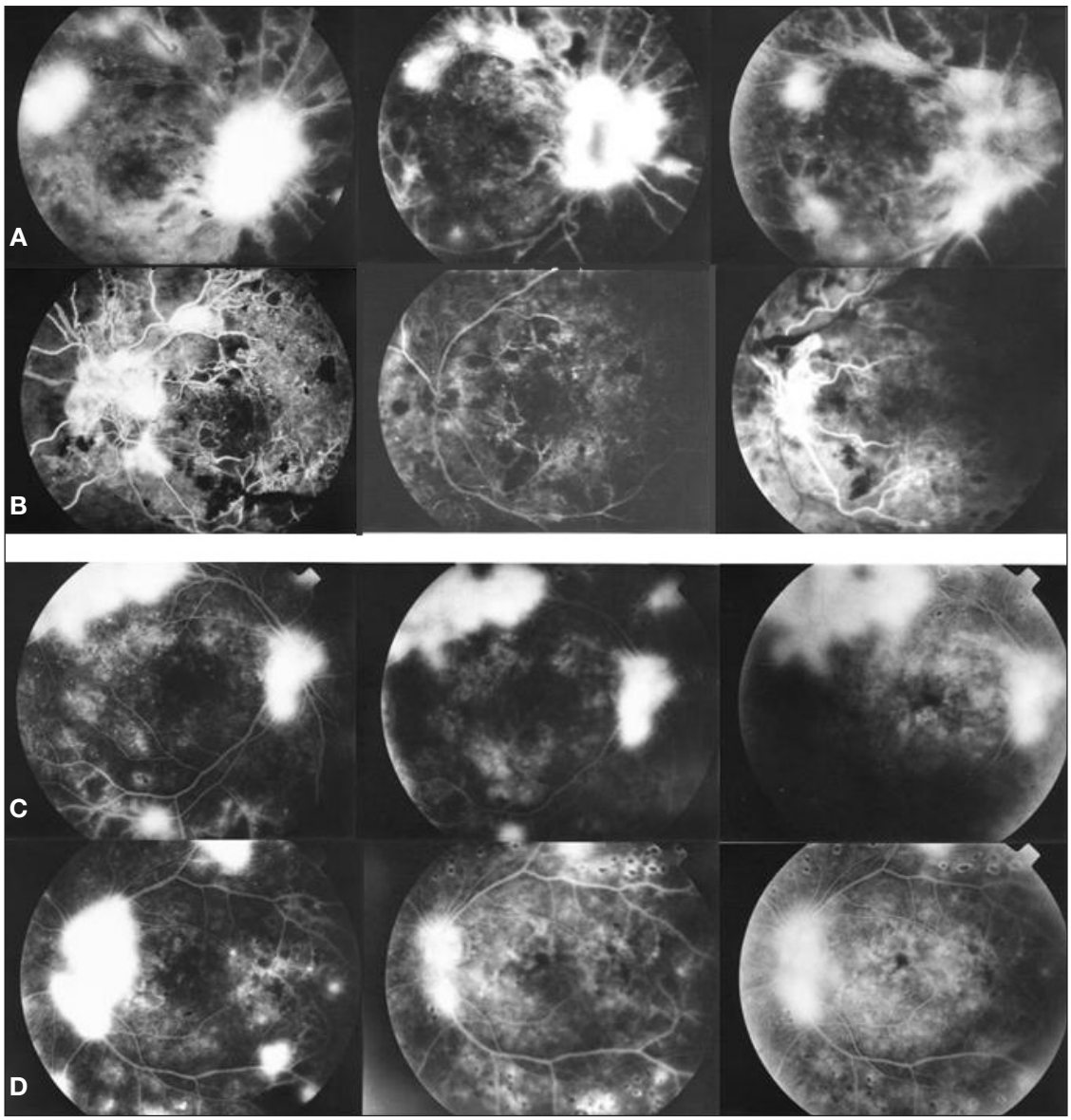

Fig. 1 - *Recurrence of proliferative diabetic retinopathy in retinal fluorescein angiography images of fellow eyes in two subjects. Right eyes (***A-C***) belong to panretinal laser photocoagulation–only group and generally did not show regression. Left eyes showed complete (upper,* **B***) or partial (lower,* **D***) regression at week 6 follow-up (from left baseline images to middle week 6 images) only to experience recurrence at week 16 (right column images).*

DISCUSSION

Bevacizumab's additional benefit in inducing regression in the early follow-up (week 6) was impressive as all patients experienced regression (12.5% partial and 87.5% complete regression). However, at week 16, regression response in the two groups was essentially identical (25% complete and 70% partial regression for Avastin group, and 25% and 65% for PRP-only group) (Tab. I). These findings reveal a temporal correlation between drug injection and decrease in active leakage from new vessels. This is compatible with the previous reports of regression of retinal neovascularization following anti-VEGF therapy in experimental and human studies (6, 7, 10), but as mentioned, the response was not sustained.

Recurrence of PDR does not seem to be related to the applied type of anti-VEGF. A variety of anti-VEGF inhibitors, namely pegaptanib (10, 12), ranibizumab (19-21, 27), and bevacizumab (13-18), have been used in the management of ocular conditions associated with neovascularization and have been shown to significantly suppress choroidal or retinal endothelial cell proliferation. However, with the currently established

TABLE II - DETERMINANTS OF PROLIFERATIVE DIABETIC RETINOPATHY RECURRENCE IN THE AVASTIN SUBGROUP

	Univariate analysis		Significance in logistic regression
	Magnitude	p value	
Age	Recurred group: 4.5 years older	0.09	0.066
Gender	Females: 5.1 times more at risk	0.03	0.112
Hypertension	Hypertensive cases: 6.9 times more at risk	0.02	0.160
Hemoglobin A1c	1.5% higher for the recurred group	0.004	0.033
Hyperlipidemia	No more risk	1.0	Not applicable

doses, none was found to have superior efficacy over the others (28).

The dose cannot explain PDR recurrence either, as we observed an impressive regression response initially. It was demonstrated that a 25 µg dose of bevacizumab has a terminal half-life of 4.32 (29) to 5.6 (30) days in the rabbit vitreous cavity after injection. The human vitreous cavity volume is three times greater than that of a rabbit (13), therefore, a dosage of 400 µg for a rabbit eye could be considered equivalent to the current study's dose of 1250 µg for the human vitreous. Following a period of 6 weeks, about 1/200 (2 µg) of the initial dose is expected to remain in the rabbit vitreous cavity. Avery et al have shown that retinal neovascularization in diabetics responds to very low doses of bevacizumab (6.2 µg) injected into the eye (14). So the early regression response observed at week 6 in this study occurs well beyond the half-life of the agent and/or its minimum effective dose.

Diabetes is a dynamic metabolic state, and the inciting factors in the pathogenesis of retinopathy and new vessel formation are not eliminated following our interventions. The relevance of this argument is substantiated by the observation that poorer systemic diabetes control, i.e. worse HbA1c levels (Tab. II), was highly correlated with the recurrence of PDR. So, continued stimulation for new vessel formation should cause recurrence. The role of systemic factors in controlling PDR and the stability of the response to PRP has been proven (31).

It may be argued that, since the severity of high-risk PDR was not quantified, the two eyes of an individual patient may not have had the same degree of PDR severity, and so the groups may not have been comparable. However, HRC-PDR in itself is a fairly uniform clinical status in the wide spectrum of diabetic retinopathy; fellow eye pair matching of the study sample and randomization of the fellow eyes for the intervention are the standard approaches to ensure comparability. Additionally, the eyes' regression responses were determined in comparison to their baseline states (and not in comparison to fellow eye, which might not have an identical HRC-PDR state).

Our study highlights the importance of the application of a comprehensive approach in managing diabetic retinopathy, i.e., tight systemic control along with conventional laser therapy. Anti-VEGF therapy, although it is yet in its infancy, is expected to revolutionize the management of retinal vascular diseases including diabetic retinopathy, should improve our clinical goals and targets substantially, and assume a significant role in the comprehensive approach mentioned. Design and implementation of a variety of anti-VEGF dosing (multiple and/or periodic injections) and delivery (including sustained release) are worthy of further investigation. Even preemptive (for non-HRC-PDR) or prophylactic (for non-PDR) anti-VEGF injections could be contemplated. Intravitreal anti-VEGF injections for patients with hazy media (vitreous hemorrhage) may halt the retinopathy progression, clear the media sooner (15), and/or facilitate vitrectomy. This approach may also be a good temporizing alternative for debilitated cases who cannot tolerate PRP and/or vitrectomy.

In conclusion, single intravitreal bevacizumab injection can strikingly augment the effect of standard laser treatment in HRC-PDR, but the response is short-lived. Return to baseline response is dependent on systemic

factors; most importantly HbA1c levels. The effects of Avastin may be different between cases of tightly controlled diabetes and poorly controlled ones. Alternative dosing of Avastin, e.g., multiple injections – along with optimized systemic control – might bring about persistently higher rates of PDR regression.

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ACKNOWLEDGEMENTS

The authors thank Dr. Amir-Hosein Mahmoudi and Dr. Shiva MehrAvaran for their critical review of the manuscript. The study was the fellowship thesis of Dr. Ramak Roohipoor.

The authors do not have competing interest.

REFERENCES

1. Kempen JH, O'Colmain BJ, Leske MC, et al. The prevalence of diabetic retinopathy among adults in the United States. Arch Ophthalmol 2004; 122: 552-63.
2. Adamis AP, Miller JW, Bernal MT, et al. Increased vascular endothelial growth factor levels in the vitreous of eyes with proliferative diabetic retinopathy. Am J Ophthalmol 1994; 118: 445-50.
3. Early Treatment Diabetic Retinopathy Study Research Group. Fundus photographic risk factors for progression of diabetic retinopathy. ETDRS report number 12. Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1991; 98: 823-33.
4. Patz A. Clinical and experimental studies on retinal neovascularization. XXXIX Edward Jackson Memorial Lecture. Am J Ophthalmol 1982; 94: 715-43.
5. Aiello LP, Avery RL, Arrigg PG, et al. Vascular endothelial growth factor in ocular fluid of patients with diabetic retinopathy and other retinal disorders. N Engl J Med 1994; 331: 1480-7.
6. Tolentino MJ, McLeod DS, Taomoto M, Otsuji T, Adamis AP, Lutty GA. Pathologic features of vascular endothelial growth factor-induced retinopathy in the nonhuman primate. Am J Ophthalmol 2002; 133: 373-85.
7. Tolentino MJ, Miller JW, Gragoudas ES, et al. Intravitreous injections of vascular endothelial growth factor produce retinal ischemia and microangiopathy in an adult primate. Ophthalmology 1996; 103: 1820-8.
8. Adamis AP, Shima DT, Tolentino MJ, et al. Inhibition of vascular endothelial growth factor prevents retinal ischemia associated iris neovascularization in a nonhuman primate. Arch Ophthalmol 1996; 114: 66-71.
9. Aiello LP, Pierce EA, Foley ED, et al. Suppression of retinal neovascularization in vivo by inhibition of vascular endothelial growth factor (VEGF) using soluble VEGF-receptor chimeric proteins. Proc Natl Acad Sci USA 1995; 92: 10457-61.
10. Adamis AP, Altaweel M, Bressler NM, et al. Macugen Diabetic Retinopathy Study Group. Changes in retinal neovascularization after pegaptanib (Macugen) therapy in diabetic individuals. Ophthalmology 2006; 113: 23-8.
11. Gragoudas ES, Adamis AP, Cunningham ET Jr, Feinsod M, Guyer DR. VEGF Inhibition Study in Ocular Neovascularization Clinical Trial Group. Pegaptanib for neovascular age-related macular degeneration. N Engl J Med 2004; 351: 2805-16.
12. Cunningham ET Jr, Adamis AP, Altaweel M, et al. Macugen Diabetic Retinopathy Study Group. A phase II randomized double-masked trial of pegaptanib, an anti-vascular endothelial growth factor aptamer, for diabetic macular edema. Ophthalmology 2005; 112: 1747-57.
13. Jorge R, Costa RA, Calucci D, Cintra LP, Scott IU. Intravitreal bevacizumab (Avastin) for persistent new vessels in diabetic retinopathy (IBEPE study). Retina 2006; 26: 1006-13.
14. Avery RL, Pearlman J, Pieramici DJ, et al. Intravitreal bevacizumab (Avastin) in the treatment of proliferative diabetic retinopathy. Ophthalmology 2006; 113: 1695.e1-15.
15. Spaide RF, Fisher YL. Intravitreal bevacizumab (Avastin)

treatment of proliferative diabetic retinopathy complicated by vitreous hemorrhage. Retina 2006; 26: 275-8.
16. Arevalo JF, Wu L, Sanchez JG, et al. Intravitreal bevacizumab (Avastin) for proliferative diabetic retinopathy: 6-months follow-up. Eye 2007; Sep 21; [Epub ahead of print].
17. Avery RL. Regression of retinal and iris neovascularization after intravitreal bevacizumab (Avastin) treatment. Retina 2006; 26: 352-4.
18. Arevalo JF, Fromow-Guerra J, Quiroz-Mercado H, et al. Primary intravitreal bevacizumab (Avastin) for diabetic macular edema: results from the Pan-American Collaborative Retina Study Group at 6-month follow-up. Ophthalmology 2007; 114: 743-50.
19. Campochiaro PA. Targeted pharmacotherapy of retinal diseases with ranibizumab. Drugs Today (Barc) 2007; 43: 529-37.
20. Emerson MV, Lauer AK. Emerging therapies for the treatment of neovascular age-related macular degeneration and diabetic macular edema. BioDrugs 2007; 21: 245-57.
21. Chun DW, Heier JS, Topping TM, Duker JS, Bankert JM. A pilot study of multiple intravitreal injections of ranibizumab in patients with center-involving clinically significant diabetic macular edema. Ophthalmology 2006; 113: 1706-12.
22. Diabetic Retinopathy Study Research Group. Photocoagulation treatment of proliferative diabetic retinopathy. Clinical application of Diabetic Retinopathy Study (DRS) findings, DRS Report Number 8. Ophthalmology 1981; 88: 583-600.
23. Rogell GD. Incremental panretinal photocoagulation. Results in treating proliferative diabetic retinopathy. Retina 1983; 3: 308-11.
24. Vander JF, Duker JS, Benson WE, Brown GC, McNamara JA, Rosenstein RB. Long-term stability and visual outcome after favorable initial response of proliferative diabetic retinopathy to panretinal photocoagulation. Ophthalmology 1991; 98: 1575-9.
25. Blankenship GW. A clinical comparison of central and peripheral argon laser panretinal photocoagulation for proliferative diabetic retinopathy. Ophthalmology 1988; 95: 170-7.
26. Flynn HW, Chew EY, Simons BD, Barton FB, Remaley NA, Ferris FL. Pars plana vitrectomy in the Early Treatment Diabetic Retinopathy Study. ETDRS report number 17. The Early Treatment Diabetic Retinopathy Study Research Group. Ophthalmology 1992; 99: 1351-7.
27. Rosenfeld PJ, Brown DM, Heier JS, et al, MARINA Study Group. Ranibizumab for neovascular age-related macular degeneration. N Engl J Med. 2006; 355: 1419-31.
28. Spitzer MS, Yoeruek E, Sierra A, et al. Comparative antiproliferative and cytotoxic profile of bevacizumab (Avastin), pegaptanib (Macugen) and ranibizumab (Lucentis) on different ocular cells. Graefes Arch Clin Exp Ophthalmol 2007; 245: 1837-42.
29. Bakri SJ, Snyder MR, Reid JM, Pulido JS, Singh RJ. Pharmacokinetics of intravitreal bevacizumab (Avastin). Ophthalmology 2007; 114: 855-9.
30. Mordenti J, Thomsen K, Licko V, et al. Intraocular pharmacokinetics and safety of a humanized monoclonal antibody in rabbits after intravitreal administration of a solution or a PLGA microsphere formulation. Toxicol Sci 1999; 52: 101-6.
31. Doft BH, Blankenship G. Retinopathy risk factor regression after laser panretinal photocoagulation for proliferative diabetic retinopathy. Ophthalmology 1984; 91: 1453-7.