

Antivascular Endothelial Growth Factor in Diabetic Retinopathy

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Abstract

Diabetic macular edema (DME) and proliferative diabetic retinopathy (PDR) represent the most common causes of vision loss in patients affected by diabetes mellitus. Diabetic retinopathy (DR) needs special attention because of its high public health impact and impact on quality of life of patients. Actually, laser retinal photocoagulation is the standard of care for the treatment of DR. However, laser treatment reduces the risk of moderate visual loss by approximately 50%, without a remarkable vision recovery. Thus, new approaches in the treatment of DR have been taken into account and, more specifically, the therapy employing antivascular endothelial growth factor (anti-VEGF) drugs could play a meaningful role. VEGF is a pluripotent growth factor that functions as an endothelial cell-specific mitogen and vasopermeability factor. Through these mechanisms VEGF plays a critical role in promoting angiogenesis and vascular leakage. A high level of VEGF has been detected in eyes presenting DME and PDR, and thereby VEGF is an attractive candidate as therapeutic target of pharmacological treatment in the management of DR. In the current chapter, the concepts and results of anti-VEGF therapy in the treatment of the DME and PDR are presented.

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Diabetic retinopathy (DR) is considered the most frequent vascular disorder being detectable in about 40% of diabetic patients 40 years and older [1]. Today, DR is the leading cause of acquired blindness among young adults throughout the developed countries [2]. Population-based epidemiological studies have estimated that after 20 years, DR is recognized to a certain extent, and that after 30 years a proliferative DR is present in the 70% of patients with diabetes mellitus type 1 [3]. The World Health Organization estimates that about 171 million persons are affected by diabetes with an expected doubling of prevalence expected in the next 20 years [4].

Role of the Vascular Endothelial Growth Factor in Diabetic Retinopathy

Hyperglycemia is the main factor involved in the pathogenesis of DR. It results in the production of glycation end products, activation of the polyol pathway, and altered transduction of cellular signals [5–7]. The following damage to endothelial cells and pericytes, through activation of oxidative and inflammatory mechanisms, produces diabetic microangiopathy affecting small-caliber retinal vessel [8]. These alterations result in the deregulation of the mechanism of flow control with subsequently hypoxia and accumulation of fluid in the retinal tissue. Hypoxia represents the likely major inducer of vascular endothelial growth factor (VEGF) gene transcription, but the overexpression of VEGF is also upregulated in response to high glucose, protein kinase C activation, and glycation end products, all elements characterizing the impairment of glycometabolic control [5–7, 9].

VEGF is a pluripotent growth factor that functions as an endothelial cell-specific mitogen and vasopermeability factor and through these mechanisms the VEGF plays a critical role in promoting angiogenesis and vascular leakage [10–13]. In DR, the impairment of the blood retinal barrier and the increased permeability are responsible for the diabetic macular edema (DME) and several investigations underline the active role of VEGF. By disrupting the intercellular tight junctions between the retinal endothelial cells, VEGF increases the extracellular accumulation of fluid from the intravascular compartment [8]. Moreover, VEGF shows a role in mediating active intraocular neovascularization.

Elevations of VEGF levels in ocular fluids from human patients with tissue hypoxia and active neovascularization secondary to DR have been well documented [14]. The increased levels of VEGF decline when treatment with panretinal photocoagulation induces regression of neovascularization. Thus, these studies demonstrated a temporal correlation between VEGF elevations and active proliferative retinopathy evidencing the role of VEGF as a key mediator of intraocular neovascularization secondary to DR. In essence, VEGF is an attractive candidate as therapeutic target of pharmacological treatment in the management of DR.

Anti-VEGF Therapy in the Treatment of Diabetic Macular Edema and Proliferative Diabetic Retinopathy

The VEGF molecular family includes five members: placental growth factor, VEGF-A, VEGF-B, VEGF-C and VEGF-D [15]. Each of the different factors may link one or more of three VEGF receptors. Moreover, between the different factors, VEGF-A plays a major role in angiogenesis and vascular permeability. Alternative splicing of VEGF gene produces nine VEGF-A isoforms (VEGF₁₂₁, VEGF₁₄₅, VEGF₁₄₈, VEGF₁₆₂, VEGF₁₆₅, VEGF_{165b}, VEGF₁₈₃, VEGF₁₈₉, VEGF₂₀₆) and among them VEGF₁₆₅ is the most abundantly expressed isoform and is detected as being mainly responsible for DR.

The pathway enclosed between VEGF gene transcription and the activation of the VEGF receptor is the object of a new therapeutic approach based on the use of the VEGF antagonist. Pegaptanib, ranibizumab, bevacizumab and VEGF Trap are molecules that are able to directly bind the VEGF protein. A new and interesting therapeutic approach is the employment of bevasiranib. This molecule, interfering with messenger RNA, interrupts the synthesis of the VEGF protein. Last of all, rapamycin, employed commonly as an immunosuppressive, anti-inflammatory or antimycotic drug, reduces the activity of VEGF molecules interfering with the promoting signal, the active synthesis of VEGF, and reduces the response of endothelial cells to VEGF.

Ranibizumab

Ranibizumab is an antigen-binding fragment (F_{ab}) derived from a humanized anti-VEGF antibody and this F_{ab} inhibits all biologically active isoforms and active proteolytic fragments of VEGF-A. Currently, ranibizumab is approved by the Food and Drug Administration for the treatment of neovascular age-related macular degeneration.

Chun et al. [16] reported the first pilot study exploring the effects of two dosing regimens of ranibizumab in eyes affected by clinically significant DME. Of 10 patients enrolled, 5 received 0.3 mg ranibizumab and 5 received 0.5 mg ranibizumab at baseline and at 1 and 2 months. At month 3, 40% of patients gained more than 15 letters, 50% gained more than 10 letters, and 80% obtained an improvement of at least 1 letter in best corrected visual acuity (BCVA). At month 3, the mean decrease in central retinal thickness was 45.3 and 197.8 μm in the low- and high-dose groups, respectively. Intravitreal injections of ranibizumab were generally well tolerated and no systemic adverse events were reported.

Nguyen et al. [17] investigated the role of ranibizumab in DME in the open-label study READ-1 (Ranibizumab for Edema of the Macula in Diabetes: Phase 1). Ten patients with chronic DME received intraocular injections of 0.5 mg ranibizumab at baseline and at 1, 2, 4, and 6 months. The main outcome measures were changes in BCVA, central retinal thickness as assessed by optical coherence tomography (OCT) measurement at the 7-month examination. Mean and median values of BCVA improved at 7 months by 12.3 and 11 letters respectively. Compared to the baseline, mean foveal thickness showed a meaningful reduction decreasing from 503 to 257 μm with a 85% reduction of the excess foveal thickness present at baseline. The injections were well tolerated with no ocular or systemic adverse events.

More recently, the results of the READ-2 study were reported. READ-2 was a prospective, randomized, interventional, multicenter clinical trial designed to compare ranibizumab with focal/grid laser, alone or in combination, in DME [18]. 126 patients were randomized to receive 0.5 mg ranibizumab, focal/grid laser photocoagulation or a combination of 0.5 mg ranibizumab and focal/grid laser. Group 1, 42 patients, received 0.5 mg ranibizumab at baseline and months 1, 3, and 5. Group 2, 42 patients,

received focal/grid laser photocoagulation at baseline and month 3 if needed (center subfield thickness was $>250\text{ }\mu\text{m}$). Group 3, 42 patients, received a combination of 0.5 mg ranibizumab and focal/grid laser at baseline and month 3. The primary outcome was the change in BCVA at month 6 in comparison with baseline. At month 6, the group receiving ranibizumab alone showed a significant improvement in mean BCVA with respect to the patients receiving focal/grid laser. The group receiving combined therapy was not statistically different from groups 1 or 2. Resolutions of 50, 33, and 45% of excess foveal thickening were assessed in groups 1, 2, and 3, respectively. The RESOLVE study was specifically designed to evaluate the efficacy and safety of ranibizumab 0.5 mg in patients with visual impairment due to DME. The RESOLVE trial (a randomized, double-masked, multicenter, phase 2 study assessing the safety and efficacy of two concentrations of ranibizumab compared with non-treatment control for the treatment of DME with center involvement) evaluated the effect of ranibizumab on retinal edema and visual acuity (VA) in 151 patients with clinically significant DME. Patients with a central macular thickness of $\geq 300\text{ }\mu\text{m}$ were randomized to receive three monthly injections with either 0.3 or 0.5 mg ranibizumab or placebo. After the three monthly intravitreal injections, treatment was administered on *pro re nata* basis for 9 months. The primary endpoint in the 1-year study was visual function at 6 months. The study design allowed the investigator to double the dose of ranibizumab if after 1 month the resolution of macular edema was incomplete. Moreover, retinal photocoagulation could be administered if needed.

The preliminary results were partially presented at the 2009 ARVO Meeting [19]. During the 12-month follow-up period, mean BCVA increased and mean CRT decreased continuously over time. The mean change in BCVA from baseline to the 12-month examination was -1.4 letters in the sham group. The groups receiving 0.3 and 0.5 mg gained, respectively, 11.8 and 8.8 letters. In order to provide further clarity on the effectiveness of treatments based on administration of steroidal or anti-VEGF drugs in comparison to conventional laser treatment, the DRCR net has designed a randomized, multicenter clinical trial which addressed the effects on visual acuity and on central retinal thickness in four groups receiving, respectively, intravitreal ranibizumab alone or associated with laser photocoagulation or triamcinolone associated with the laser treatment or laser treatment alone.

The study recruited 691 patients and examined a total of 854 eyes in a follow-up period of 2 years. Two hundred ninety-three eyes were randomized to received laser alone, 187 eyes were assigned to the group receiving 0.5 mg ranibizumab + prompt laser, 188 eyes received 0.5 mg ranibizumab + deferred laser (at least 24 weeks), and 186 eye were included in the group receiving 4 mg intravitreal triamcinolone + prompt laser.

At 1-year examination, the mean change in the visual acuity letter score respect to the baseline value showed a statistically significant improvement in the ranibizumab + prompt laser group ($+9\pm 11$ letters) and ranibizumab + deferred laser group ($+9\pm 12$) but not in the triamcinolone + prompt laser group ($+4\pm 13$) compared with the laser group ($+3\pm 13$).

Over the 2 years of follow-up a different correlation between visual acuity change and retinal thickness was observed in each group. A progressive reduction in mean central subfield thickness was noted in the laser group during the 24 months of follow-up; however, the mean change in visual acuity did not continue to increase from the 1- to 2-year visit as noted instead during the first year of follow-up.

In the triamcinolone + laser group, during the first year of follow-up an improvement of visual function was associated with a significant reduction in CST whereas from the 1- to 2-year examination the mean CST increased in parallel with a visual acuity reduction.

Ranibizumab groups showed a parallel visual acuity improvement associated with a CST reduction from baseline to 12-month visit and following the OCT results remained relatively stable up to 24-month examination and paralleled the visual acuity outcomes during this time.

Intraocular hypertension and cataract surgery were more frequently noted in the triamcinolone + prompt laser group in comparison to groups receiving ranibizumab + laser or laser alone.

The current large prospective randomized clinical trial confirms the preliminary promising results in the treatment of DME and suggests as a combined therapy might offer a more efficacious approach in this disorder where the multi-factorial pathogenesis involves several processes [20].

Actually, several multicenter international clinical trials (e.g. RESTORE, RIDE and RISE) are ongoing in order to evaluate the efficacy and safety of ranibizumab 0.5 mg as monotherapy or in combination with laser photocoagulation in eyes affected by DME.

With regard to the effects of ranibizumab on proliferative diabetic retinopathy (PDR), no studies are available in the literature evaluating this topic. However, DRCR.net has designed a prospective, randomized, comparative clinical trial to evaluate the role of ranibizumab or triamcinolone intravitreal injection as adjunctive treatment to panretinal photocoagulation for PDR.

Pegaptanib

Pegaptanib is a pegylated 28-nucleotide RNA aptamer that binds to the VEGF_{164/165} isoform at high affinity. VEGF₁₆₅ levels are present in human eyes affected by DR with increased concentration and play an active role in promoting angiogenesis and in enhancing vascular permeability. Initially, pegaptanib was employed only in the treatment of neovascular age macular degeneration, where it obtained the approval of the Food and Drug Administration. Considering the role of VEGF₁₆₅ in DR and the safety and tolerability profile of intravitreally administered pegaptanib, a phase II trial was specifically designed to investigate the effects of pegaptanib in the management of DME.

The Macugen Diabetic Retinopathy Study was a randomized, sham-controlled, double-masked, dose-finding phase II trial designed to evaluate the effect of three doses of intravitreal pegaptanib vs. sham injection in patients affected by clinically significant DME [21]. The patients were randomized to receive 0.3, 1.0 or 3.0 mg of pegaptanib or sham injection at baseline, week 6 or week 12. If needed, further injections were administered every 6 weeks up to a maximum of three additional injections. Retinal laser photocoagulation could be delivered if the investigators judged it to be necessary. The main outcome measures were changes in BCVA, central retinal thickness as assessed by OCT measurement, and additional therapy with photocoagulation between weeks 12 and 36. At the final visit at week 36, the group of patients receiving pegaptanib 0.3 mg was significantly superior to sham injection, as measured by mean change in VA (+4.7 vs. -0.4 letters; $p = 0.04$), proportions of patients gaining >10 letters of VA (34 vs. 10%; $p = 0.003$), change in mean central retinal thickness (68 μm reduction vs. 3.7 μm increase; $p = 0.02$). Moreover, only 25% of patients receiving pegaptanib required retinal photocoagulation in comparison with 40% of patients receiving a sham injection ($p = 0.04$). It is noteworthy that patients receiving 1.0 or 3.0 mg did not show a significant improvement compared to 0.3 mg with regard to BCVA or CRT changes. In general, pegaptanib was well tolerated at various concentrations; endophthalmitis occurred in 1 of 652 injections and was successfully treated without severe visual loss.

The Macugen Diabetic Retinopathy Study also provided new information on the ability of pegaptanib sodium to lead to regression of retinal neovascularization in proliferative DR [22]. Of 16 patients having retinal neovascularization in the study eye at baseline, 13 were assigned to receive pegaptanib treatment and 3 were assigned to sham injections. At 36 weeks, 8 of 13 (62%) in the pegaptanib treatment group showed regression of neovascularization, as assessed by fundus photography or fluorescein angiography, whereas no such regression occurred in 3 sham-treated eyes. In 3 of 8 with regression, neovascularization progressed at week 52 after cessation of pegaptanib at week 30, suggesting the necessity of repeated injections to control retinal neovascularization.

More recently, Gonzalez et al. [23] reported the results of a prospective, randomized, controlled, open-label, exploratory study designed to compare the efficacy of intravitreal pegaptanib vs. panretinal laser photocoagulation (PRP) in the treatment of active PDR. 20 subjects with active PDR were assigned at a 1:1 ratio to receive pegaptanib treatment in 1 eye every 6 weeks for 30 weeks or with PRP. In 90% of eyes randomized to pegaptanib, retinal neovascularization showed a complete regression by week 3. By week 12, in all eyes receiving pegaptanib a complete regression of retinal proliferation was obtained and preserved through week 36. In the PRP-treated group, at the 9-month examination, 25% demonstrated complete regression, 25% showed partial regression, and 50% showed persistent active PDR.

With regard to BCVA and although the difference between the groups was not statistically significant ($p = 0.22$), pegaptanib-treated eyes showed an increase of 5.8 letters in BCVA at 36 weeks, whereas the PRP-treated eyes lost 6.0 letters.

Bevacizumab

Bevacizumab is a full-length recombinant humanized antibody active against all isoforms of VEGF and currently it is approved for the treatment of metastatic colon cancer. However, anti-VEGF treatment approved for wet AMD, ranibizumab and pegaptanib is restricted in many countries; this situation has induced many retinal specialists to off-label use of bevacizumab and contemporarily it has allowed to underline the role of this VEGF antagonist in many retinal disorders including neovascular AMD, macular edema in non-ischemic central retinal vein occlusion, pseudophakic cystoid macular edema and DR.

Short-term effects of bevacizumab for DME in a large randomized phase II clinical trial were initially reported by the Diabetic Retinopathy Clinical Research Network [24]. 109 subjects with DME and Snellen acuity equivalent ranging from 20/32 to 20/320 were prospectively enrolled and randomized to 1 of 5 groups: (a) focal photocoagulation at baseline, (b) intravitreal injection of 1.25 mg bevacizumab at baseline and 6 weeks, (c) intravitreal injection of 2.5 mg bevacizumab at baseline and 6 weeks, (d) intravitreal injection of 1.25 mg bevacizumab at baseline and sham injection at 6 weeks, or (e) intravitreal injection of 1.25 mg bevacizumab at baseline and 6 weeks with photocoagulation at 3 weeks.

BCVA in groups receiving bevacizumab alone showed a median one-line improvement at the 3-week visit, which was preserved up to 12 weeks and was greater than the change in the group receiving only focal photocoagulation at baseline. A similar trend was observed with regard to central retinal thickness; comparing focal photocoagulation vs. bevacizumab alone, a greater reduction in central retina thickness was observed in the bevacizumab groups at 3 weeks. Subsequently, only a trend towards a greater reduction at 6, 9, and 12 weeks was detected.

No significant differences among groups receiving bevacizumab 1.25 vs. 2.5 mg for changes in BCVA or central retinal thickness were observed. Moreover, no meaningful differences were found comparing bevacizumab groups with groups receiving combined treatment in reduction in central subfield thickening or improvement in VA. Also, Lam et al. [25] evaluated the efficacy of two dosing regimens of bevacizumab at the 6-month follow-up. 48 patients were randomized to receive three monthly intravitreal injections of 1.25 or 2.5 mg bevacizumab. At each monthly scheduled visit a significant mean central foveal thickness reduction was observed in both groups. Similarly, mean logMAR BCVA showed a statistically significant improvement from baseline to the final visit at 6 months (from 0.63 to 0.52 in the 1.25-mg group and from 0.60 to 0.47 in the 2.5-mg group). No significant difference in BCVA was observed between the two groups. Moreover, the study confirmed the effects of bevacizumab when the intravitreal injection reached a plateau of action at 3 weeks with a subsequent decline, and repeated injections were necessary to maintain the initial effect.

More recently, Arevalo et al. [26] reported the results of a retrospective, multicenter, interventional, comparative case series with a long-term follow-up that extended to

24 months. The study evaluated 139 eyes that underwent an intravitreal injection of 1.25 or 2.5 mg bevacizumab. The main outcome measures were central foveal thickness as measured by OCT and changes in BCVA. Additional injections were administered if recurrence of macular edema was detected on OCT associated with VA loss.

At 1 month, both groups showed a statistically significant improvement in BCVA and subsequently the gain was preserved up to the 24-month examination. The 1.25-mg group improved from 20/150 to 20/107 at 1 month, and to 20/75 at 24 months. In the 2.5-mg group, BCVA improved from 20/168 to 20/118 at 1 month, and to 20/114 at the final visit. OCT examination evidenced a good anatomical response in both groups. At 1 month, the mean central macular thickness measurements decreased significantly from 446 to 333 μm ; during the subsequent period a similar trend was observed up to 24 months with a final mean value of 279.7 μm . Similar results were observed in the 2.5-mg group. Over the 24-month follow-up period, 807 injections were performed and the mean number of injections per eye was 5.8 (range 1–15) at a mean interval of 12.2 ± 10.4 weeks.

Long-term efficacy of repeated injections of intravitreal 1.25 mg bevacizumab for the treatment of chronic diffuse DME was also reported by Kook et al. [27]. The study included 126 patients affected by chronic, diffuse, clinically significant DME in part not responsive to previous treatments including laser photocoagulation (62% focal laser treatment, 38% panretinal laser treatment), triamcinolone intravitreal injection (41%) or vitrectomy (11%). 67 and 59 patients completed the scheduled visits to 6 and 12 months, respectively. At the 6-month examination, logMAR BCVA ranged from a baseline value of 0.82 to 0.74 considering all patients. The mean BCVA of patients who completed the 12-month follow-up improved similarly from 0.82 to 0.74. Mean central retinal thickness decreased from 463 to 374 μm after 6 months, and to 357 μm after 12 months with a statistically significant difference. The authors concluded that even in cases with chronic diffuse ischemic DME not responding to other therapies, a successful treatment with repeated intravitreal injections of bevacizumab can be observed in a long-term follow-up.

Other studies compared intravitreal bevacizumab treatment with intravitreal triamcinolone or focal retinal photocoagulation in refractory DME or as primary treatment. Paccola et al. [28] designed a randomized, prospective study in order to evaluate the anatomical response and VA outcomes after a single intravitreal injection of 4 mg triamcinolone acetonide or 1.25 mg bevacizumab in the refractory diffuse DME. The study enrolled 26 patients; at baseline, logMAR BCVA was 0.936 and 0.937 in the triamcinolone and bevacizumab groups, respectively. At 6 months, BCVA improved to 0.91 and 0.92 without achieving a significant difference; however, interim analysis at the 1-, 2- and 3-month examinations evidenced a significant improvement in the triamcinolone group in comparison with the bevacizumab group. Central macular thickness was significantly reduced in the intravitreal triamcinolone group compared with the bevacizumab group at weeks 4, 8, 12 and 24. The analysis of changes in CMT over the follow-up showed a significant from baseline

at weeks 4, 8 and 12 in the triamcinolone group and at weeks 4 and 8 in the bevacizumab group.

A similar prospective and comparative case series was reported by Shimura et al. [29]. The study recruited 14 patients with bilateral long-standing DME; in each patient, 1 eye was selected to receive a single intravitreal injection of triamcinolone (4 mg) and the other to receive a single intravitreal bevacizumab injection (1.25 mg).

logMAR BCVA in the triamcinolone group improved significantly from 0.64 to 0.33 at 1 week, and the gain was subsequently preserved up to 12 weeks. At a final observation period of 24 weeks, BCVA decreased to 0.47 but was still significantly different from the baseline value. Similarly, BCVA in the bevacizumab group improved from 0.61 to 0.39 at 1 week and maintained the initial gain up to 4 weeks. At 12 weeks, BCVA returned to the initial level. No further decrease or improvement was observed in the following 3 months. Between the two groups, a statistically significant difference of BCVA was observed in favor of the triamcinolone group at 3 and 6 months.

Morphological analysis revealed a significant decrease of foveal thickness from 522 to 342.6 μm in the triamcinolone group at 1 week. At 12 weeks the foveal thickness maintained the improvement, but at 6 months it increased slightly to 410.4 μm . The bevacizumab group showed a significant foveal thickness reduction at 1 week from 527 to 397 μm and preserved the improvement also at 4 weeks. In the following weeks, the foveal thickness showed a progressive worsening and reached the initial level at 12 weeks; no further worsening or improvement was observed in the following 12 weeks. Between the two groups, a statistically significant difference of foveal thickness was observed in favor of the triamcinolone group at 1, 3 and 6 months.

A more recently, randomized, three-arm clinical trial comparing intravitreal bevacizumab injection (1.25 mg) alone or in combination with intravitreal triamcinolone acetate (2 mg) versus macular laser photocoagulation as a primary treatment of DME was published by Soheilian et al. [30]. Each arm enrolled 50 patients receiving the treatment at baseline and at 12 weeks as needed. The main outcome measure was change in BCVA at the 4-month examination, but the study also provided the results at 36 weeks. The bevacizumab group showed a significant BCVA improvement from 0.71 to 0.54 at 6 weeks; the initial gain was maintained at each following visit at 12, 24 and 36 weeks. The patients who underwent to combined treatment showed a significant BCVA improvement from 0.73 to 0.60 at 6 weeks. This group maintained BCVA also at 12 weeks but lost the statistically significant improvement at 6 and 9 months. In the macular photocoagulation group, BCVA showed a stabilization at 6 weeks in comparison to the baseline value (0.60 vs. 0.55) and similar values were observed at all follow-up visits; however, we should consider that the three groups differed with regard to the baseline VA values.

The mean values of central macular thickness decreased significantly in all groups only at 6 weeks in comparison to the baseline values and although the reduction was greater in the bevacizumab group with respect to the other two groups, no statistically significant differences were registered at any of the follow-ups among the three groups. Retreatment was administered to 27 eyes up to 6 months and specifically 14

eyes received an additional bevacizumab injection, 10 eyes received combined treatment and macular photocoagulation was performed in 3 eyes.

Another interesting element of this study is represented by regression of retinal neovascularization that presented initially in 9 patients. After the first bevacizumab treatment, the neovascularization resolved in all subjects. Treatment of retinal neovascularization associated with DR has been the object of study in several investigations exploring the employment of bevacizumab as an antiangiogenic drug.

In a retrospective study, Avery et al. [31] investigated the biologic effects of intravitreal bevacizumab in patients with retinal and iris neovascularization secondary to diabetes mellitus in 45 eyes. The patients received intravitreal bevacizumab in a dose-escalating regimen (6.2 g to 1.25 mg). Changes in fluorescein angiographic leakage and in BCVA were the primary and secondary outcome, respectively. At the 1-week examination, fluorescein angiography disclosed a complete or at least partial reduction in leakage of the neovascularization. Recurrence was registered in variable time: in 1 case recurrence of retinal neovascularization was detected after 2 weeks whereas in the other cases no recurrent leakage was registered at the last follow-up of 11 weeks.

In a prospective, non-randomized open-label study, Jorge et al. [32] evaluated the effects of bevacizumab in patients affected by active proliferative DR refractory to laser treatment and with a BCVA value inferior to 20/40. Each patient received a single intravitreal bevacizumab injection of 1.5 mg. 15 patients completed the scheduled visits up to 12 weeks of follow-up. Mean logMAR BCVA improved significantly from 0.90 at baseline to 0.76 at week 1; subsequently the improvement was preserved up to the 12-week examination. At baseline the mean neovascularization leakage area was 27.79 mm². At the 1- and 12-week examinations, the mean neovascularization leakage area decreased significantly to 5.43 and 5.50, respectively.

Additional data on the use of bevacizumab in patients affected by proliferative DR complicated by vitreous hemorrhages has been provided by Moradian et al. [33]. 38 patients were enrolled and prospectively followed up to 20 weeks. Mean logMAR BCVA increased from 1.13 to 0.86 1 week after the injection; a further improvement was observed at 6, 12 and 20 weeks, with a final mean value of 0.53. Vitreous hemorrhages resolved significantly at the 1- and 12-week examinations and about 50% of patients showed a complete resolution. At 20 weeks, only the 23% of the eyes presented a slight degree of vitreous hemorrhages.

A more recent investigation corroborates these results. Huang et al. [34] evaluated the efficacy of intravitreal bevacizumab combined with panretinal photocoagulation in the treatment of PDR complicated by vitreous hemorrhage. In their prospective study, 40 patients underwent an intravitreal bevacizumab injection (1.25 mg) followed by PRP. An additional injection was administered if no signs of decreased vitreous hemorrhages were noted. In the event of persistent vitreous hemorrhage over 12 weeks, vitrectomy was performed. The vitreous clear-up time and rate of vitrectomy were registered and compared with the control group treated with conventional methods. The vitreous clear-up time in the bevacizumab group was significantly

lower than in the control group (11.9 vs. 18.1 weeks). Similarly, the bevacizumab group required vitrectomy in 10% of the patients compared to 45% of the control group. 31 and 9 patients respectively received 1 and 2 injections.

VEGF Trap

The VEGF Trap-Eye (Regeneron Inc.) is a 115-kDa recombinant fusion protein of portions of VEGF receptors 1 and 2, and the Fc region of human IgG which binds all VEGF-A isoforms with higher affinity in comparison to other anti-VEGFs, including bevacizumab and ranibizumab [35]. Moreover, VEGF Trap-Eye has a longer half-life in the eye after intraocular injection and it binds other members of the VEGF family including placental growth factors 1 and 2, which have been shown to contribute to excessive vascular permeability. This higher affinity will probably allow to employ lower doses and to maintain a longer duration of action [36, 37].

A study phase I exploring the safety and bioactivity of a single injection of 4.0 mg VEGF Trap-Eye in subjects with DME has been reported by Do et al. [38]. At 4 weeks, 5 of the 5 subjects showed a good anatomical response with a meaningful reduction in excess retinal foveal thickness. All but 1 subject maintained the reduction at 6 weeks after the injection. The median improvement in BCVA was 9 letters on the ETDRS chart at 1 month and 3 letters at 6 weeks. In general, a single intravitreal injection of 4.0 mg VEGF Trap-Eye was well tolerated and no serious ocular adverse events were reported. An extended phase II study on a larger sample of patients and with a longer follow-up is waited with great interest.

Bevasiranib

Small interfering RNA (siRNA) molecules are able to inactivate the RNA messenger and suppress RNA translation. Bevasiranib is a specific siRNA designed to decrease the level and activity of VEGF mRNA. In this regard, bevasiranib may have a role in the treatment of DR [39–41].

The RACE trial was a double-masked, randomized, phase 2 study, designed to investigate the safety and efficacy of bevasiranib in patients with DME [42]. At the end of the follow-up, 48 patients had completed the scheduled visit. Each patient was assigned to one of three arms receiving respectively bevasiranib 0.2, 1.5 or 3.0 mg. Bevasiranib was administered monthly for 3 months. Primary and secondary outcome measures were changes on macular edema as measured by OCT and on BCVA. In this pilot study, there was a trend showing a decrease in macular thickness between weeks 8 and 12, where the higher doses result in a larger reduction in thickness than the lowest dose. BCVA showed a stabilization in 91% of patients. No severe side effect was observed, however 4 patients reported mild uveitis.

Rapamycin

Rapamycin is a macrocyclic antibiotic produced by the bacterium *Streptomyces hygroscopicus* and found in the soil of Easter Island and it was initially classified as a macrolide fungicide; nevertheless, it shows a wide range of actions including immunosuppressive, antitumor and antiangiogenic properties. Rapamycin binds specifically the FKBP12; the active complex inhibits the mammalian target of rapamycin (mTOR), a kinase, which integrates growth factor-activated signals including signals promoting angiogenesis mediated by VEGF. Moreover, mTOR is an activator of hypoxia-inducible factor 1 α , which upregulates the transcription of VEGF. In hypoxic cells, rapamycin can interfere with HIF-1 α activation by increasing the rate of its degradation [43–45].

In essence, the antiangiogenic properties of rapamycin are associated with a decrease in VEGF production and a reduction in the response of vascular endothelial cells to stimulation by VEGF. Rapamycin may therefore have a meaningful role as therapy for retinal disorders characterized by pathological vascular permeability and proliferation. Preliminary results of application of rapamycin for DME were presented at the 2008 ARVO Meeting by Blumenkranz et al. [46]. A multicenter, open-label phase 1 dose-escalation study was designed with the aim of evaluating the safety and pharmacological activity of rapamycin. 50 patients were randomly assigned to receive either a single intravitreal (44, 110, 176, 264, or 352 μ g) or a single subconjunctival (220, 440, 880, 1,320, or 1,760 μ g) rapamycin injection. The primary outcome was changes in BCVA evaluated on the ETDRS chart and changes on central retinal thickness evaluated by OCT at 14, 45, 90 and 180 days. With regard to BCVA, a significant improvement was observed at 14, 45 and 90 days in both groups receiving a 440- μ g subconjunctival injection and the group receiving a 352- μ g intravitreal injection. Specifically, the intravitreal injection group showed a mean gain of 11.6, 6.4, and 7.8 letters at 14, 45 and 90 days. The subconjunctival injection group gained 8.8, 11.4, and 7.4 letters. A significant central macular thickness reduction was observed in both groups with mean OCT reductions of 33, 78, and 54 μ m in the patients receiving a subconjunctival injection and 72, 42, and 61 μ m in the patients receiving an intravitreal injection. No dose-limiting toxicities were observed and no serious ocular adverse events were registered.

Conclusion

Anti-VEGF therapy has opened a new perspective in the treatment of DME and of proliferative retinopathy. However, the pathogenesis and the course of the DR will demand a more complex approach. Currently, retinal laser photocoagulation is the only recommended treatment in the clinically significant macular edema and in PDR. These statements are derived from large clinical randomized, controlled trials which are not available for the pharmacological classes analyzed above [47]. For each molecule, anti-VEGF must be defined as the more effective scheme of administration in

reference to the characteristics of the single molecules, the dosing, and for some also the routes of administration; moreover, the use of selective vs. non-selective molecules has to be better defined. A better definition of anti-VEGF treatment in reference to the characteristics and stadiation of DR is necessary; thus, more specific inclusion and exclusion criteria are required including the presence of early or advanced DR, duration of symptoms and signs, visual function, presence of concomitant retinal edema and proliferation, and previous treatments. A head-to-head comparison between anti-VEGF and conventional laser therapy is necessary, and, considering the multifactor pathogenesis, verification of the effectiveness of combined treatments, including laser treatment and corticosteroid therapy, is needed.

Finally, the surgical approach could receive additional support in the management of complications of advanced DR, neovascular glaucoma and in the progression of macular edema associated with cataract surgery.

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