Preoperative Intravitreal Bevacizumab Use as an Adjuvant to Diabetic Vitrectomy: Histopathologic Findings and Clinical Implications

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Purpose: To evaluate the effects of intervals between preoperative intravitreal injection of bevacizumab (IVB) and surgery on the components of removed diabetic fibrovascular proliferative membranes.

Design: Interventional, consecutive, prospective, comparative case series.

Participants: A total of 52 eyes of 49 patients with active diabetic fibrovascular proliferation with complications necessitating vitrectomy.

Methods: Participant eyes that had IVB were divided into 8 groups in which vitreoretinal surgery was performed at days 1, 3, 5, 7, 10, 15, 20, and 30 postinjection. A group of eyes with the same diagnosis and surgical intervention without IVB injection was used for comparison. In all eyes, proliferative membrane specimens obtained during vitrectomy were sent for histopathologic examination using hematoxylin-eosin stain, immunohistochemistry (CD34 and smooth muscle actin), and Masson’s trichrome stain.

Main Outcome Measures: Comparative analysis of different components of the fibrovascular proliferation (CD34, smooth muscle actin, and collagen) among the study groups.

Results: Pan-endothelial marker CD34 expression levels starting from day 5 postinjection were significantly less than in the control group (P < 0.001), with minimum expression at or after day 30 postinjection. Positive staining for smooth muscle actin was barely detected in the control eyes at day 1, and consistently intense at day 15 and beyond (P < 0.001). The expression level of trichrome staining was significantly high at day 10, compared with control eyes (P < 0.001), and continued to increase at subsequent surgical time points.

Conclusions: A profibrotic switch was observed in diabetic fibrovascular proliferation after IVB, and our results suggest that at approximately 10 days post-IVB the vascular component of proliferation is markedly reduced, whereas the contractile components (smooth muscle actin and collagen) are not yet abundant.

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An optimal balance between obtaining the maximal antiangiogenic effect of bevacizumab while minimizing the untoward effect of TRD progression is critical in determining the optimal duration between bevacizumab injection and surgical intervention. Needless to say, this ideal goal is not an easy task to achieve in a limited clinical trial. Studying histopathologic changes in the surgically removed fibrovascular membranes might have a predictive value regarding the proper surgical time point to obtain a favorable outcome from the adjunctive use of IVB.

This study evaluated the effect of the interval between preoperative IVB and surgery on the different components of the surgically removed diabetic fibrovascular proliferative membranes and the possible clinical implications of this evaluation.

Patients and Methods

Fifty-two eyes of 49 patients with active PDR (defined as the presence of PDR with active fibrovascular proliferation) were included in this study. All eyes were scheduled for vitrectomy. Indications for surgery included TRD involving the macula, extramacular TRD with vitreous hemorrhage (VH), and persistent VH in patients who have recent clinical evidence of active fibrovascular proliferation before the onset of hemorrhage. We excluded from the study all patients who received any previous anti-VEGF injections or scatter laser treatment during the last 6 months, patients with TRD due to fibrous proliferation without active new vessels, patients who had previous vitrectomies, and patients with uncontrolled systemic hypertension or recent history of thromboembolic events (myocardial infarction or cerebrovascular accident).

The study was approved by the local institutional review board at Magrabi Eye and Ear Center (Saudi Arabia) and the ophthalmology department at Benha University (Egypt), which follows the tenets of the Declaration of Helsinki. Informed consent was obtained from each patient after an explanation of the benefits and hazards of each procedure.

Surgical Technique

Standard 3-port pars plana vitrectomy with an endothelial dissection of the epiretinal membrane (ERM) was performed by 3 experienced vitreoretinal surgeons at 3 different centers (Magrabi Eye and Ear Centers at Damman, Saudi Arabia and Sana'a, Yemen, and Benha University Hospital, Benha, Egypt) with ERM obtained during vitrectomy. These membranes were immediately fixed in 10% neutral buffer formalin and then submitted for histologic examination at King Fahd University Hospital, Dhahran, Saudi Arabia.

Specimen Preparation

After fixation, ERM were dehydrated and paraffin-embedded blocks were made using standard techniques. Serial 4-μm tissue sections were cut and placed on positively charged glass slides and dried overnight at 56°C. One set of sections was used for routine hematoxylin–eosin staining, and parallel sections were used for immunohistochemistry (CD34 and smooth muscle actin), and Masson’s trichrome stain for collagen. Immunohistochemical staining was performed using the Ventana automated slide stainer (Ventana Medical Systems, Inc., Tucson, AZ) with all histology procedures performed using appropriate immunohistochemical controls. Various components of the fibrovascular membranes were studied, namely, angiogenic activity determined by the immune detection of pan-endothelial marker CD34, the myofibroblastic activity by immune detection of smooth muscle actin expression, and the fibrous component by Masson’s trichrome stain for collagen.

Angiogenesis Grading

All estimations were done in a masked manner. For microvessel density estimation, each slide was first scanned at ×100 to determine “hot spots,” defined as areas with the maximum number of microvessels. The slides were then examined at ×400. Microvessels were counted in each of the hot spots at ×400. Large vessels were excluded. Areas of staining with no discrete breaks were counted as a single vessel. The presence of a lumen was not required. The microvessel density estimation was estimated by determining the average number of vessels in each of the hot spots and expressing the result as the number of vessels per ×400 high-power field; on the basis of that number, each slide was assigned an angiogenesis grade from + to ++ +++. The entire stained sample was considered when assigning the angiogenesis grade; this grading was based solely on visual assessment.

Assessment of Smooth Muscle Cell Proliferation

Intensity of the immune staining was evaluated semiquantitatively.

Assessment of Fibrous Tissue Proliferation

Intensity of the trichrome staining for collagen was evaluated semiquantitatively and scored in 4 grades from + to + + + + .

Statistical Analysis

Data were analyzed using 1-way analysis of variance with postBonferroni test.

Results

Of the 49 patients studied, 24 were female and 25 were male; their ages ranged from 24 to 73 years, with a mean age of 54.82 years. Nineteen of these patients had type 1 diabetes mellitus, and 30 patients had type 2 diabetes mellitus. Of the 52 eyes undergoing surgery, 26 had surgery for VH with extramacular TRD, 12 had surgery for macular traction, and 13 had surgery for persistent VH, and 5 had surgery for tractional rhegmatogenous retinal detachment.

Clinical Observations

In some cases in which clear preinjection and preoperative photos and ocular coherence tomography pictures could be obtained, it was clearly evident that the amount of vascularity of the proliferative membranes was reduced with prominence of the fibrous component (Figs 1 [available at http://anajournal.org] and 2).
Moreover, ocular coherence tomography showed progression of the traction with contraction of the ERM in 2 of 32 eyes (6.3%), leading to partial separation from the retinal surface in 1 patient (No. 24, Fig 2) and severe macular traction with lamellar hole formation in 1 patient (No. 47, Fig 3 [available at http://aojournal.org]).

Pan-endothelial Marker CD34
Pan-endothelial marker CD34 expression levels starting from day 5 post-injection were significantly less than in the control group ($P < 0.001$) and remained consistently low (+ or less) starting from day 10 post-injection, with minimum expression (+) in all specimens removed at or after day 30 post-injection (Table 1, available at http://aojournal.org; Figs 4 and 5).

Smooth Muscle Actin
Positive staining for smooth muscle actin was barely detected in the control and day 1 groups. Significant expression levels (compared with control) were observed at post-injection day 3 and beyond ($P < 0.05$) and became consistently intense from day 15 post-injection onward ($P < 0.001$). Maximum staining intensity (++++) was observed at day 30 and beyond (Table 2, available at http://aojournal.org; Figs 4 and 5).

Trichrome Staining for Collagen
All specimens had positive trichrome staining for collagen. However, significant expression levels (compared with control) were observed at day 10 and beyond ($P < 0.001$). Staining became consistently intense from day 20 post-injection and beyond (Table 3, available at http://aojournal.org; Figs 4 and 5).

Interpretation of the histopathologic findings shows that, at day 10 post-IVB injection, neovascularization was significantly reduced; whereas the contractile elements of the membrane (smooth muscle actin and collagen) were still at low levels (Fig 5).

Discussion

Vascular endothelial growth factor has been implicated in the development of PDR, among other factors, but was considered the most important angiogenic factor in PDR. Anti-VEGF agents have been effectively used for reduction of neovascularization related to PDR and to decrease intraoperative bleeding, allowing for better visualization and dissection of ERM with less incidence of iatrogenic breaks. This was evidenced in our study by the significant reduction in neovessels expressing the pan-endothelial marker CD34, which was noted from day 5 post-injection and became consistently low from day 10 onward. Avery et al. noted that some large-caliber neovessels remained perfused 1 week after IVB but did not leak fluorescein dye. Intraoperative bleeding from such large-caliber vessels might be troublesome. In our study, specimens obtained at days 1 and 3 post-injection showed no significant reduction in the level of expression of CD34 from that observed in the control group. These findings suggest that early operative intervention within a few days of IVB might not provide the required favorable adjunctive effect of its use.

Easy separation of ERM from the retinal surface noted by Chen and Park, as well as surgeons in our study, could be explained by the contraction of the ERM that maintained attachments to the retina at the epicenters with more tractional force exerted on the retina. This facilitates the removal of ERM; however, if surgery is delayed or these increasing traction forces cannot be monitored it might endangering the macula, central vision, or final visual outcome by progression of TRD. Thus, it is noteworthy that before injecting IVB, every effort should be made to prepare the patient medically for urgent surgical intervention should the need arise; moreover, if a pa-
An angio-fibrotic switch hypothesis has been proposed by Kuiper et al. to help in understanding the complex multifactorial process of fibrovascular proliferation. The hypothesis describes the critical balance between VEGF and CTGF as a major determinant of the nature of the fibrovascular proliferation. According to this hypothesis, the marked decrease in the activity of VEGF (whether caused by a decrease in VEGF level from the ablated ischemic areas after pan-retinal photocoagulation or by VEGF blockade by anti-VEGF drugs) causes a switch of proliferative process into fibrogenesis. This could provide an explanation for the histologic findings in our study, the clinical findings of Arevalo et al. and Wu et al., the previously reported TRD after pan-retinal photocoagulation by Lütke et al. and the recently reported development of TRD within 8 weeks post-indirect pan-retinal photocoagulation for PDR by Tinley and Gray.

In an in vitro endothelial cell proliferation assay by Wang et al., the minimum bevacizumab concentration required to completely block the VEGF-A induced endo-
Fig. 5. Mean values of positive staining (scored 0–4) for different components (pan-endothelial marker CD34, smooth muscle actin, and tenascin stain) for collagen of fibrovascular membranes of patients in the control group and at different time points after preoperative IVB in the treatment groups. At 10 days post-IVB, neovascularization was significantly reduced whereas the contractile elements of the membranes (smooth muscle actin and collagen) were still at low levels. IVB = intravitreal injection of bevacizumab.

Vascular leakages were maintained for 48 days. After clearance of leakages, recurrence of leakage in fluorescein angiograms showing more or less stain leakage was noted in 14 of 15 patients (93%). Tenascin was not yet abundant; however, in the presence of dense fibrovascular proliferation, it is advisable to closely monitor patients for the untoward progression of TRD after IVB. Our histologic findings are in accordance with many published clinical findings and might be predictive of an optimal time interval for the preoperative use of adjunctive IVB, which makes surgery more successful with less intraoperative bleeding and complications, consequently leading to a better visual outcome; however, such favorable outcomes require larger-scale clinical trials to be proven.

References