The Effect of Photocoagulation in Ischemic Areas to Prevent Recurrence of Diabetic Macular Edema After Intravitreal Bevacizumab Injection

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Submitted: April 29, 2014
Accepted: June 30, 2014

Citation: Takamura Y, Tomomatsu T, Matsumura T, et al. The effect of photocoagulation in ischemic areas to prevent recurrence of diabetic macular edema after intravitreal bevacizumab injection. *Invest Ophthalmol Vis Sci*. 2014;55:4741–4746. DOI: 10.1167/iovs.14-14682

Purpose. This study aimed to investigate whether targeted retinal photocoagulation (TRP) for nonperfused areas (NPAs) could have a preventive effect on the recurrence of diabetic macular edema (DME) after intravitreal injection of bevacizumab (IVB).

Methods. Eyes in the IVB group received 1.25 mg IVB, and eyes in the IVB+TRP group received 1.25 mg IVB combined with TRP of NPAs. Two weeks before IVB administration, grid/focal photocoagulation (PC) had been performed in both groups. After IVB treatment, the best corrected visual acuity (BCVA) and central retinal thickness (CRT), determined by optical coherence tomography, were measured every month for 6 months.

Results. Fifty-two patients with DME were enrolled and randomized to an IVB group ($n = 26$) and an IVB+TRP group ($n = 26$). After IVB, the CRT decreased temporally, and the CRT significantly increased at 2 months and thereafter in the IVB group but did not increase significantly in the IVB+TRP group. Maximum increase in CRT after IVB was significantly correlated with the width of NPAs in the IVB group ($P = 0.0368$), but not in the IVB+TRP group. Best corrected visual acuity in the IVB+TRP group was significantly better than that in the IVB group 5 and 6 months after treatment ($P < 0.05$).

Conclusions. Targeted retinal photocoagulation for NPAs was effective to maintain the reduced CRT after grid/focal PC and IVB for patients with DME. These results suggest that retinal ischemia is associated with the pathogenesis of recurrence of DME after IVB. (www.umin.ac.jp/ctr number, UMIN000007566.)

Keywords: diabetic macula edema, bevacizumab, targeted retinal photocoagulation

Diabetic macular edema (DME) is the most common cause of visual impairment in patients with diabetes. Focal laser therapy is currently the standard treatment, but diffuse DME does not respond well to grid lasers.[1] The limited results obtained using grid/focal photocoagulation (PC) have prompted interest in other alternative or adjunct treatments for DME, including anti-vascular endothelial growth factor (VEGF) therapy[2–4] and steroids,[5–7] either alone or in combination with laser treatment.[8–10]

A potent activator of angiogenesis, VEGF enhances collateral vessel formation and increases the permeability of the microvasculature.[11] A marked increase in VEGF expression has been found in the vitreous and aqueous fluids in patients with proliferative diabetic retinopathy (PDR) and DME.[12–14] Recent prospective, randomized studies have demonstrated the efficacy of intravitreal injections of anti-VEGF drugs[4,8–10] in reducing retinal thickness and improving visual acuity.

Although the use of anti-VEGF drugs is increasingly prevalent, the therapeutic effect seems to be transient, and retinal neovascularization and macular swelling tend to recur after a single injection of anti-VEGF drugs.[15] Thus frequent intravitreal injections of anti-VEGF drugs are required to control macular edema, which then raises the possibility of adverse events such as endophthalmitis and retinal detachment. Therefore it is clinically important to find techniques to reduce the recurrence of DME after intravitreal injection of anti-VEGF drugs to avoid the need for repeated injections.

Vascular endothelial growth factor is generally recognized to be released from hypoxic or ischemic retinas. Recently, the significant association between the degree of DME and width of nonperfused areas (NPAs) indicates that retinal ischemia is associated with the pathogenesis of DME.[16] Although panretinal photocoagulation (PRP) is the gold standard as a treatment for widely spread NPAs implying severe retinal ischemia,[17] the side effects of PRP treatment include permanent retinal scarring, resulting in scotomas and decreased peripheral night vision.[18] Recently, targeted retinal photocoagulation (TRP) has been designed to treat areas of retinal capillary NPAs and intermediate retinal ischemic zones in PDR; this may spare better-perfused tissue from laser-induced tissue scarring.[19] Against this background, we designed this randomized clinical trial to determine the effect of peripheral TRP for NPAs on the recurrence of DME after intravitreal injection of bevacizumab (IVB).

Patients and Methods

This clinical trial was approved by the University of Fukui Institutional Review Board and complied with the tenets of the

Declaration of Helsinki. The protocol and the safety and efficacy implications of the interventions were explained to all participants before enrollment. All patients provided informed consent. This study was registered with the University Hospital Medical Information Network Clinical Trials Registry (UMIN-CTR) of Japan (ID UMIN 000007566; date of access and registration, April 15, 2012).

Patient Eligibility and Exclusion Criteria

Patients with type 2 diabetes who had a reduction in best corrected visual acuity (BCVA) between 20/40 and 20/320 due to DME were eligible for this clinical trial. The criterion for thickening of the central macula was defined as a central retinal thickness (CRT) of $\geq$250 μm in the central subfield, based on Cirrus optical coherence tomography (OCT; Carl Zeiss Meditec, Dublin, CA, USA). When leakage from capillary retinal vessels and microaneurysms, corresponding to macular edema, was confirmed and the presence of peripheral NPAs was identified by fluorescein angiography (FA), patients were enrolled in the study. During angiography, seven standard fields (7SF) were taken by experienced photographers with a Kowa VX-10i fundus camera (Kowa Ltd., Nagoya, Japan). A montage was created manually from the fields. The number of pixels in NPAs was measured on FA images stored in the image filing system Claio (PSC, Inc., Ehime, Japan) by Adobe software (Photoshop CS6 Extended; Adobe Systems, Inc., San Jose, CA, USA) and divided by the pixels in the area of the optic disc. The major exclusion criteria included (1) grid/focal PC, PRP within the previous 12 months; (2) active intraocular inflammation or infection in either eye; (3) uncontrolled glaucoma in either eye; (4) previous treatment with the antiangiogenic drug used in this study; (5) a history of stroke; and (6) a systolic blood pressure (BP) $>$ 160 mm Hg or a diastolic BP $>$ 100 mm Hg, or untreated hypertension.

Study Protocol

Patients were randomized using a 1:1 allocation ratio to either grid/focal laser treatment and IVB (the IVB group) or combination treatment with grid/focal lasers and IVB followed by TRP for NPAs (the IVB+TRP group). The research investigator was not involved in the randomization process. Although the patients and study physicians were not blinded to the therapeutic modality, the OCT technician, the study optometrist, and all statisticians were blinded to the patient randomization. If both eyes were eligible for the study, the eye with the worse BCVA was treated first and enrolled in the study.

Eligible patients initially underwent grid/focal laser treatment using the Early Treatment Diabetic Retinopathy Study (ETDRS) protocol with some modifications.[20] Focal treatment was administered to leaking microaneurysms, and grid treatment was administered to thickened retinas and NPAs between 500 and 3000 μm from the center of the fovea. Two weeks after grid/focal laser treatment, 1.25 mg bevacizumab (Avastin; Genentech, Inc., South San Francisco, CA, USA) was injected intravitreally in 0.05 mL with a 30-gauge needle through the supratemporal quadrant under sterile conditions. Standard procedures for injections included application of topical anesthetic, insertion of a lid speculum, and cleaning of the conjunctiva with povidone-iodine. One week after IVB, patients in the IVB+TRP group underwent PC in peripheral ischemic areas using retinal photocoagulator MC-300 (NIDEK Co., Ltd., Aichi, Japan). Targeted retinal photocoagulation of NPAs was performed under the following conditions: (1) NPAs confirmed by FA were selectively coagulated; (2) laser power was 200 mW, duration of exposure 200 ms in yellow wavelength (577 nm); (3) coagulation spot size was adjusted to 200 μm with a space the size of approximately one coagulation spot between each of the spot parts; and (4) when multiple NPAs were present close to one another, coagulation of the entire area containing the NPA was acceptable. Photocoagulation was carried out by one trained ophthalmologist (MG).

Central retinal thickness was measured using OCT on the day of grid/focal PC and IVB and every month for the following 6 months. Best corrected visual acuity was measured at the same time. Patients were eligible for additional IVB as a retreatment during 6 months after the first IVB if OCT CRT increased $>$ 150 μm compared with the lowest previous measurement.

A sample size of 25 subjects in each group, while accounting for an approximately 10% dropout rate, would have provided 90% power to prove, at a one-sided α level of 0.025, superiority of the combination therapy with TRP compared with IVB monotherapy for the mean change in CRT from baseline at month 6. We had chosen a minimum effect size of 80 μm for the difference of CRT between the IVB and IVB+TRP groups at month 6. The significance of differences between the two study groups in age, duration of diabetes mellitus, and level of hemoglobin A1c was analyzed by unpaired *t*-test. The significance of the difference in CRT and BCVA was analyzed using Mann-Whitney test. Bartlett's test was used to examine equal variances across samples. After inspecting the normal distribution of the data, CRT or BCVA among the different time points was compared using a repeated measures analysis of variance (ANOVA) with Dunnett's test. The variance of CRT at various time points from month 1 to 6 among the IVB group or the IVB+TRP group was tested by a repeated measures ANOVA. Thereafter, each CRT value according to time points was examined for differences from those at month 1 by Dunnett's multiple comparison test. Differences with P values $<$ 0.05 were considered statistically significant.

Results

Fifty-two eyes of 52 patients were enrolled in this study between April 15, 2012 and June 10, 2013. Twenty-six patients were randomized to the IVB+TRP group and 26 to the IVB group. One patient in the IVB group exited the study since his last visit was at 5 months after IVB and thus he lost the follow-up at 6 months (Fig. 1). The Table shows the baseline characteristics at registration for the two groups. There were no significant differences between the two groups in general medical history or ophthalmological factors. All patients in both groups had limited NPAs observed in the peripheral retina by FA (Fig. 2) and initially underwent grid/focal PC. There was no significant difference between the groups in the number of spots treated in each eye, with a mean of 26 $\pm$ 14 (SD) in the IVB group and 21 $\pm$ 18 in the IVB+TRP group.

Figure 3 shows changes in CRT in the IVB and IVB+TRP groups over time. The mean CRT dropped significantly in both groups in the 2 weeks between grid/focal PC and IVB, with the mean $\pm$ SD dropping from 519 $\pm$ 184 to 479 $\pm$ 153 μm in the IVB group and from 526 $\pm$ 176 to 495 $\pm$ 180 μm in the IVB+TRP group. At the time of IVB, no significant difference was seen in CRT between the two groups ($P = 0.3234$). During the first month after IVB, the CRT in both the IVB and IVB+TRP groups dramatically decreased to 287 $\pm$ 89 and 318 $\pm$ 127 μm, respectively. However, the CRT in the IVB group gradually increased from month 1 to 4 after IVB, and was relatively stable from month 4 to 6. Analysis by repeated measures ANOVA showed a significant variance (P $<$ 0.001) among the IVB

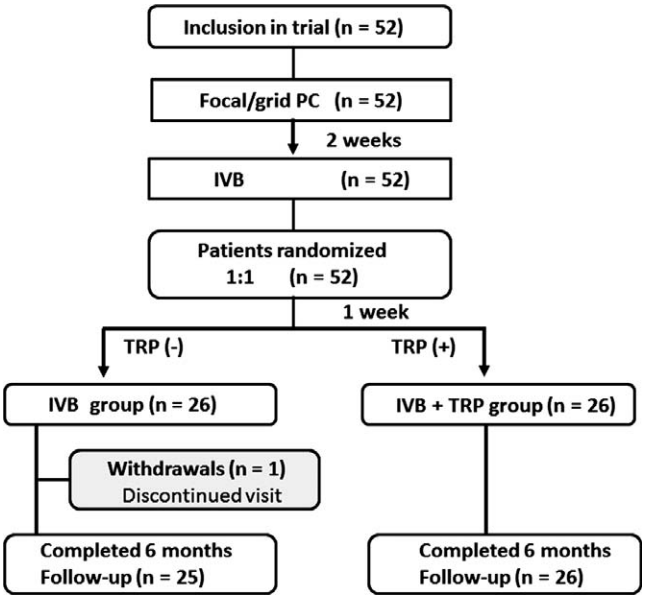

Figure 1. Diagram showing study design. Eligible eyes underwent grid/focal lasers followed by intravitreal injection of bevacizumab (IVB); then they were randomized 1:1 to receive targeted retinal photocoagulation (TRP) for ischemic lesion or no additional laser treatment.

group (months 1–6), and the analysis using Dunnett's test showed that the CRT values were significantly greater for each at month 2 through to month 6 than at month 1. In contrast, the CRT in the IVB+TRP group did not change significantly through 6 months after IVB. The CRT in the IVB group was significantly greater than in the IVB+TRP group at 3 ($P = 0.0476$), 4 ($P = 0.0052$), and 5 ($P = 0.0133$) months after IVB.

The initial BCVAs for the IVB and IVB+TRP groups were 0.808 ± 0.265 and 0.844 ± 0.289, respectively, and were not significantly different ($P = 0.5224$) (Fig. 4). Analysis by repeated measures ANOVA showed a significant variance ($P < 0.01$) in BCVA among the IVB+TRP group, and analysis using Dunnett's test showed that the BCVA values were significantly better for each at month 5 and 6 than at baseline (at the time of grid/focal PC). In the IVB group, there was no significant improvement in BCVA through the observational periods. The BCVA for the IVB+TRP group was significantly better than that of the IVB group at 5 ($P = 0.0134$) and 6 months ($P = 0.0436$) after IVB. Five and two patients in the IVB group and the IVB+TRP group, respectively, met the retreatment criteria and thus underwent additional IVB once at 5 months after the first IVB. There were no cases of infectious endophthalmitis, vitreous hemorrhage, or retinal detachment in either group, and no progression of cataracts was apparent during the 6 months of the study.

In this study, 65.4% and 61.5% of eyes in the IVB and IVB+TRP groups, respectively, had a history of PRP (Table). In each case, we defined the maximum degree of reswelling of DME by calculating the highest CRT minus the lowest CRT in the measurement after IVB. This value for eyes in the IVB group was significantly greater than in the IVB+TRP group, for both eyes with ($P = 0.0011$, compare lanes 1 and 3) and eyes without ($P = 0.0373$, compare lanes 2 and 4) a history of PRP (Fig. 5). On the other hand, significant differences were not observed between eyes in the presence and absence of a history of PRP in either the IVB group ($P = 0.1611$, compare lanes 1 and 2) or the IVB+TRP group ($P = 0.1264$, compare lanes 3 and 4). There was a significant correlation between the maximum increases in CRT after IVB and the width of NPAs in the IVB group ($P = 0.0368$, $R^2 = 0.169$), but not in the IVB+TRP group ($P = 0.6143$) (Fig. 6).

Table. Baseline Characteristics at the Time of Registration

	IVB Group,* $n = 26$	IVB+TRP Group,† $n = 26$	*P* Value
Age, y	71.3 ± 8.2	73.1 ± 8.5	0.49‡
Sex, male/female	13/13	14/12	0.78§
Duration of diabetes mellitus, y	14.4 ± 7.9	13.7 ± 6.3	0.48‡
Hemoglobin A1c, %	7.5 ± 1.6	7.3 ± 1.7	0.61‡
Insulin therapy (%)	9 (34.6%)	8 (30.8%)	0.77§
Left eye:right eye	12:14	13:13	0.78§
Phakic eyes (%)	14 (53.8%)	22 (84.6%)	0.57§
DME type			
Focal (%)	14 (53.8%)	14 (53.8%)	0.78§
Diffuse (%)	13 (50%)	13 (50%)	0.78§
Nonperfused areas, disc areas	12.9 ± 4.6	13.2 ± 4.2	0.43‡
Visual acuity, logMAR	0.81 ± 0.27	0.84 ± 0.29	0.71‡
Central retinal thickness, μm	519.4 ± 184.3	526.2 ± 176.1	0.55‡
History of PRP (%)	17 (65.4%)	16 (61.5%)	0.77§
Total number of past PC shots	1094 ± 309	1149 ± 285	0.47‡

* Intravitreal injection of bevacizumab group.
† Intravitreal injection of bevacizumab + targeted retinal photocoagulation group.
‡ Unpaired *t*-test.
§ χ^2 test.

Discussion

Intravitreal injection of bevacizumab has been shown to dramatically reduce intraocular levels of VEGF,[21,22] and data from many prospective clinical trials have demonstrated promising effects for anti-VEGF drugs as treatments for DME.[4–6,10,11] However, frequent injections have been necessary, as macular swelling can recur after IVB. A possible explanation for this is that the intravitreal half-life of 1.25 mg bevacizumab is only approximately 3 days,[22] and this short

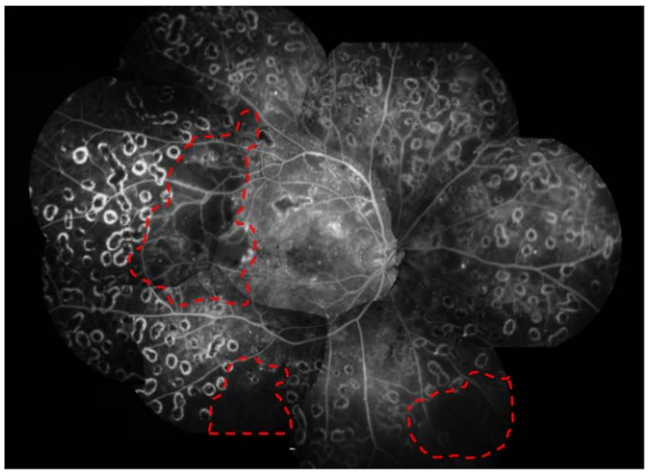

Figure 2. Fluorescein angiography image (composite image) showing the conditions for inclusion in this study. Nonperfused areas are indicated by *red broken lines*.

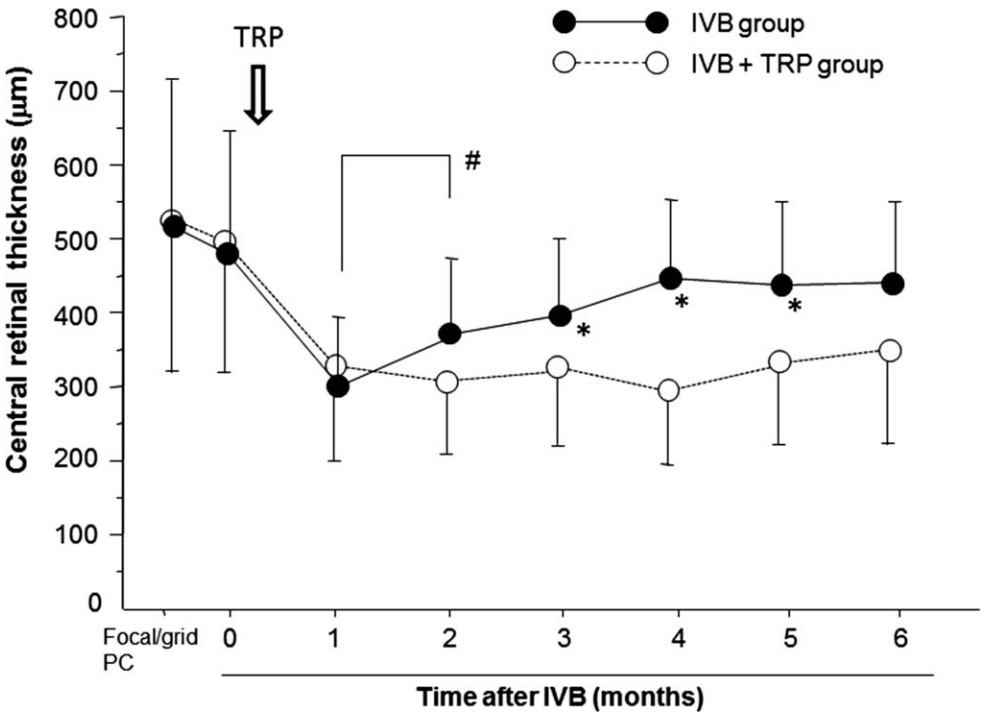

FIGURE 3. Changes in central retinal thickness after grid/focal lasers and IVB with or without TRP. Data represent mean ± standard deviation (SD). *Arrow* indicates the timing of TRP. #$P < 0.05$ (compared with CRT at month 1 after IVB); *$P < 0.05$ (IVB group versus IVB+TRP group).

half-life may result in a rapid reduction in the intraocular concentration of bevacizumab so that its therapeutic effect is transient. Another possible reason is that the ischemic areas of the peripheral retina continue to release VEGF after IVB. Even if the intraocular VEGF levels could be reduced by IVB and consequently DME improved, if the ischemic retina continues to produce VEGF and induce leakage from retinal vessels, then recurrence of macular edema would be induced. In this clinical trial, we selected patients who exhibited NPAs identified by FA, indicating the presence of hypoxia. We performed PC for NPAs

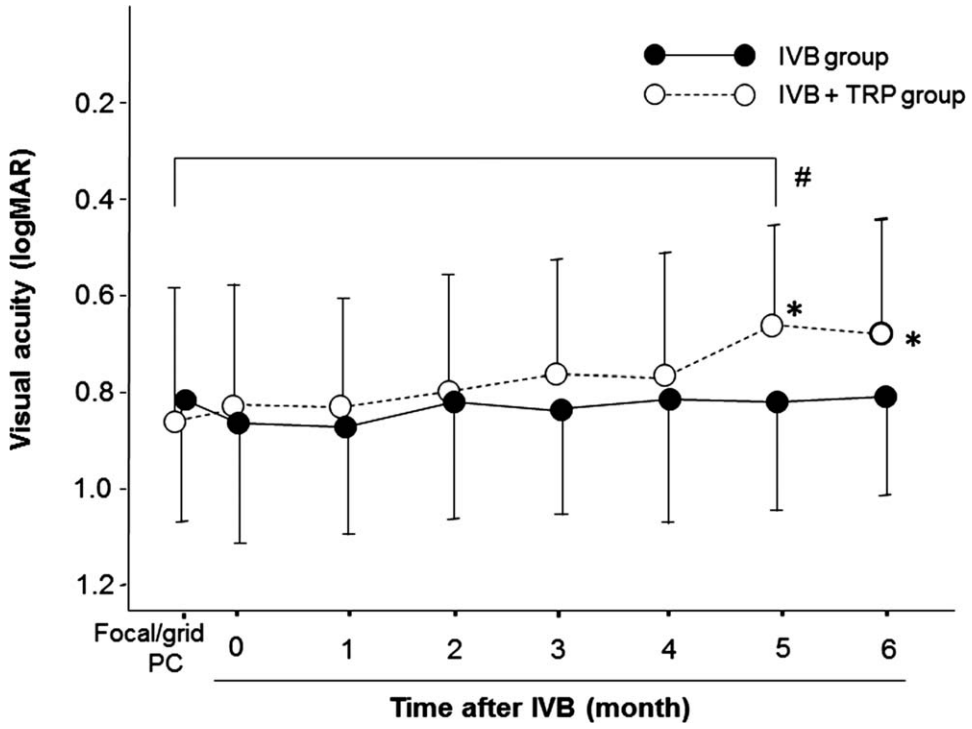

FIGURE 4. Changes in best corrected visual acuity (BCVA) (logarithm of the minimum angle of resolution [logMAR]) after grid/focal lasers and IVB with or without TRP. Data represent mean ± standard deviation (SD). #$P < 0.05$ (versus each time point); *$P < 0.05$ (IVB group versus IVB+TRP group).

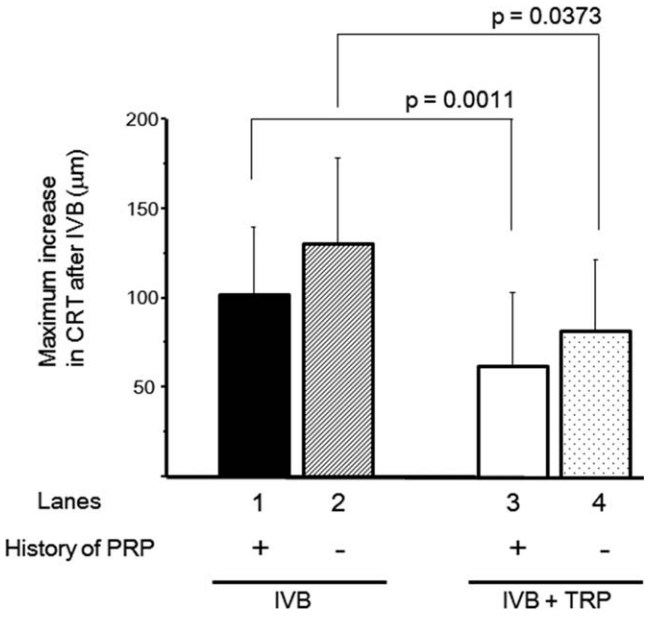

FIGURE 5. Maximum increase in central retinal thickness (CRT) after IVB with or without history of panretinal photocoagulation.

localized in peripheral retina after IVB and looked at whether this prevented the recurrence of DME. We found that TRP for NPAs inhibited the increase in CRT after IVB and improved visual acuity. This result suggests that ocular hypoxia is associated with the pathogenesis of the recurrence of DME after IVB.

Actually, we also found that the levels of reswelling of DME were significantly associated with the width of NPAs. This finding supports our hypothesis, the association of retinal ischemia with the recurrence of DME after IVB. This significant relationship was not observed in the IVB+TRP group, indicating that TRP has a potential to prevent the recurrence of DME even if NPAs were relatively wide.

The Japanese Society of Ophthalmic Diabetology showed that TRP was effective at preventing progression from non-PDR to PDR.[23] Our data indicate that TRP is also useful to prevent the recurrence of DME after IVB. Targeted retinal photocoagulation not only prevented the increase in CRT, but also improved BCVA after IVB. The largest difference in CRT between the groups was found at 4 months, while a significant difference in BCVA was noticed at 5 and 6 months. Since a significant improvement in BCVA followed the reduction in CRT, it is likely that the improved visual outcome resulted from the earlier recovery from DME. At the end of follow-up at 6 months, the difference in CRT between the groups became insignificant. The reason may be that the number of eyes that underwent additional IVB at 5 months as retreatment was greater in the IVB group (five eyes) in comparison to IVB+TRP group (two eyes). In addition, our data may imply that multiple injections are still needed to maintain the therapeutic effect of an anti-VEGF drug for a longer period even if TRP was successfully performed. To verify this issue, longitudinal study is necessary.

Matsuyama et al.[24] showed that VEGF levels in the aqueous humor were dramatically reduced by IVB and remained at a low level for up to 8 weeks after IVB when PRP was used as a pretreatment. Therefore, it is probable that the effect of additional laser contributes to the inhibition of VEGF production from ischemic areas. On the other hand, Shimura et al.[25] reported that the vitreous level of VEGF was not influenced by PRP, one possible explanation being that the vitreous levels of VEGF in PDR had been saturated before PRP, in the absence of bevacizumab injection.

This study had a limitation. We created a montage manually from 7SF; however, this technique may miss ischemic areas in the outer periphery. Compared with conventional 7SF imaging, it is reported that current ultra-widefield (UWF) retinal imaging systems, which enable capturing of up to 200° of the retina in a single image, reveal significantly more retinal vascular pathology, including NPAs and neovascularization in patients with diabetic retinopathy.[26,27] It is still unclear whether the outer peripheral area of our case series included NPAs that could not be captured by 7SF. It would be ideal to estimate the status of ischemia more widely using the UWF imaging system.

In conclusion, additional TRP for NPAs had effects to prevent the recurrence of DME after IVB, suggesting that residual retinal ischemia is associated with the pathogenesis of

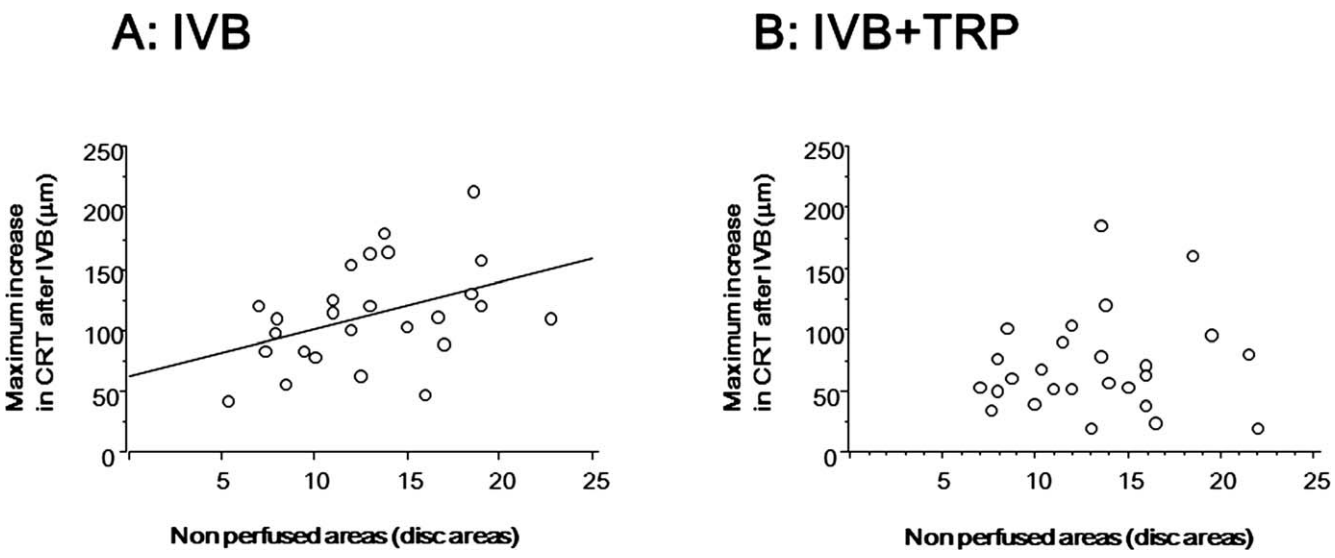

FIGURE 6. Linear correlation of maximum increase in CRT after IVB with the width of nonperfused areas in eyes of diabetic patients who underwent IVB (**A**) or IVB with TRP (**B**). There was a significant relationship in the IVB group ([**A**] $P = 0.0368$, $R^2 = 0.169$), but not in the IVB+TRP group ($P = 0.6143$).

DME. Targeted retinal photocoagulation for NPAs would contribute to reducing the frequency of IVB, the costs, visits to the clinic, and the risk of adverse events, including endophthalmitis and retinal detachment. Based on our data, we would recommend that attention be paid to ischemic lesions in the peripheral retina in the management of DME.

Acknowledgments

Supported in part by a grant-in aid for scientific research (No. 24592620) from the Japan Society for the Promotion of Science, Tokyo, Japan.

Disclosure: **Y. Takamura,** None; **T. Tomomatsu,** None; **T. Matsumura,** None; **S. Arimura,** None; **M. Gozawa,** None; **Y. Takihara,** None; **M. Inatani,** None

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