Anti-VEGF Therapy Reduces Inflammation in Diabetic Macular Edema

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Keywords

Diabetic macular edema · Ranibizumab · Inflammation · Aqueous flare value · Cytokines

Abstract

Purpose: Correlations among the aqueous flare value (an indicator of inflammation), several functional-morphologic parameters, and aqueous humor levels of multiple cytokines or inflammatory factors were investigated in patients with diabetic macular edema (DME) receiving intravitreal ranibizumab injection (IRI). **Methods:** Aqueous humor levels of 12 cytokines, growth factors, or inflammatory factors were measured in 46 DME patients who received IRI. Vascular endothelial growth factor (VEGF), soluble VEGF receptor (sVEGFR), and the other cytokines/inflammatory factors were measured by the suspension array method. In addition, aqueous flare values were measured with a laser flare meter, and central macular thickness (CMT) was examined by optical coherence tomography. **Results:** At 1 month after IRI therapy, the aqueous flare value showed a significant decrease compared with before treatment (baseline). Significant correlations were noted between the aqueous flare value and the aqueous humor levels of 6 factors/cytokines, including sVEGFR-1, placental growth factor, monocyte chemoattrac-

tant protein 1, soluble intercellular adhesion molecule-1, interleukin (IL)-6, and interferon-inducible 10-kDa protein (IP-10). There was also a significant correlation between the change in aqueous flare value and improvement in CMT 1 month after IRI. **Conclusions:** These findings suggest that IRI reduces subclinical inflammation and that the aqueous flare value is influenced by inflammatory factors/cytokines. In addition, the change in the aqueous flare value may be an indicator of the response of CMT to IRI in patients with DME.

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Introduction

Diabetic retinopathy is frequently complicated by diabetic macular edema (DME), which arises when breakdown of the blood-retinal barrier leads to increased vascular permeability [1]. DME can occur at any stage in the course of diabetic retinopathy and it is the most frequent cause of visual impairment in the developing world. Treatment of DME was dramatically altered by the introduction of anti-VEGF (vascular endothelial growth factor) agents. It has been reported that DME is improved by intravitreal injection of ranibizumab (an Fab antibody fragment that neutralizes all isoforms of VEGF-A) or

aflibercept (a recombinant fusion protein containing parts of the extracellular domains of human VEGF receptor 1 [VEGFR-1] and VEGFR-2 fused to the Fc portion of human IgG1) [2–4]. However, the macular edema sometimes persists or recurs despite patients receiving multiple intravitreal ranibizumab injections (IRIs), suggesting that other mechanisms may contribute to DME in addition to VEGF.

We recently reported a relation between the ocular fluid levels of several inflammatory factors and retinal vascular permeability, as well as the severity of DME [5, 6], suggesting that inflammation plays an important role in macular edema. This hypothesis is supported by the report that injection of triamcinolone acetonide resulted in a more significant decrease of central retinal thickness and visual acuity than ranibizumab [7], and the finding of a significantly higher aqueous flare value in DME patients compared with normal controls [5]. In addition, several studies have demonstrated the benefits of anti-VEGF therapy in improving visual acuity and decreasing retinal thickening in eyes with DME [4, 8, 9], while in 32–66% of eyes treated with at least 6 monthly injections, macular edema persisted, often with reduced visual acuity [4]. However, the relation between inflammation and the response of DME to anti-VEGF therapy remains unclear, as well as the relation between changes of the aqueous flare value and the prognosis of DME in patients receiving anti-VEGF therapy. Accordingly, we measured the aqueous flare value, several functional-morphologic parameters, and aqueous humor levels of multiple cytokines/growth factors in DME patients receiving anti-VEGF therapy and evaluated the relations among these factors. The aim of the study was to estimate the relationship between inflammation and the response to anti-VEGF therapy.

Materials and Methods

Subjects

This study was conducted at the Department of Ophthalmology of Tokyo Medical University Hachioji Medical Center. The subjects were 46 patients with DME who received IRI treatment (0.5 mg in 0.05 mL; Lucentis; Novartis, Buläch, Switzerland) between August 2014 and October 2017. Criteria for anti-VEGF therapy were: a central macular thickness (CMT) >300 µm and a best-corrected visual acuity (BCVA) <25/30. None of the patients received treatment for macular edema before this study.

The aqueous flare was measured with a laser flare meter (FC-600, Kowa Co. Ltd., Tokyo, Japan), as described previously [5]. Aqueous humor samples were obtained at the time of IRI. Under topical anesthesia, a mean volume of 0.1 mL of aqueous humor was collected by anterior chamber limbal paracentesis with a 30-gauge needle attached to an insulin syringe. The intravitreal ranibizu-

mab was then injected via the pars plana at 3.5 mm from the limbus. Antibiotic ointment was applied for 7 days after the procedure. Immediately after collection, the aqueous humor samples were transferred to sterile plastic tubes and stored at –80 °C until analysis. The aqueous humor levels of cytokines (sVEGFR-1, sVEGFR-2, VEGF, placental growth factor [PlGF]), platelet-derived growth factor (PDGF)-AA, soluble intercellular adhesion molecule-1 (sICAM-1), monocyte chemoattractant protein 1 (MCP-1), interleukin (IL)-6, IL-8, IL-12(p70), IL-13, and interferon-inducible 10-kDa protein (IP-10) were measured with enzyme-linked immunosorbent assays (xMAP; Luminex Corp. Austin, TX, USA), as reported previously [6]. The patients were evaluated 1 month after anti-VEGF therapy by complete eye examination, spectral-domain optical coherence tomography (Heidelberg Engineering, Heidelberg, Germany), measurement of vital signs, review of their medical history (including concomitant medications and concurrent ocular procedures), and an assessment of safety. BCVA was determined as the logarithm of the minimum angle of resolution (logMAR).

Exclusion criteria were: a history of glaucoma, uveitis, retinal diseases other than DME, rubeosis iridis, ocular infections, laser photocoagulation, intraocular surgery (including cataract surgery and anti-VEGF therapy), and severe kidney injury (e.g., the need for renal dialysis).

Changes of Clinical Parameters

To assess improvement in vision, the change in BCVA was calculated by subtracting the value after anti-VEGF therapy from the value before anti-VEGF therapy. For assessment of macular edema, the percent change of CMT (%ΔME) was calculated:

$$\% \Delta \text{ME} = (\text{ME}_{\text{pre}} - \text{ME}_{\text{post}}) / \text{ME}_{\text{pre}} \times 100 = (1 - \text{ME}_{\text{post}} / \text{ME}_{\text{pre}}) \times 100,$$

where ME_{pre} and ME_{post} are the extent of macular edema before and 1 month after IRI, respectively.

Statistical Analysis

Data are presented as mean ± SD, median (IQR), or *n*. The paired *t* test was employed to compare continuous variables between baseline and 1 month after anti-VEGF therapy. To examine relationships among the variables, Spearman's rank-order correlation analysis or Pearson's correlation analysis was performed, as appropriate. Statistical significance was considered at *p* < 0.05.

Results

The DME patients included 29 men and 17 women with a mean age of 65.4 ± 9.5 years. The mean duration of diabetes was 10.5 ± 4.9 years and mean hemoglobin (Hb)A_{1c} 8.0 ± 1.3%. Nine patients were using insulin (19.6%). In addition, 25/46 patients (54.3%) had hypertension and 21 (45.7%) had hyperlipidemia. Mean baseline BCVA was logMAR 0.46 ± 0.29 and it improved significantly to logMAR 0.36 ± 0.27 at 1 month after anti-VEGF therapy (*p* = 0.034; Fig. 1a). Mean CMT was 514 ± 159 µm at baseline and it decreased significantly to 299 ± 111 µm 1 month after anti-VEGF therapy (*p* < 0.001;

Fig. 1. BCVA and CMT 1 month after intravitreal ranibizumab injection. **a** Mean BCVA improved significantly from logMAR 0.46 ± 0.29 at baseline to 0.36 ± 0.27 at 1 month after anti-VEGF therapy ($p = 0.034$). **b** Mean CMT decreased significantly from $514 \pm 159 \mu\text{m}$ at baseline to $299 \pm 111 \mu\text{m}$ at 1 month ($p < 0.001$).

Fig. 1b). The aqueous flare value also showed a significant decrease 1 month after anti-VEGF therapy (from 16.3 ± 7.9 to 12.4 ± 5.3 photon counts/ms; $p < 0.001$; Fig. 2). The reduction in CMT was not significantly correlated with the improvement of visual acuity 1 month after anti-VEGF therapy ($\rho = 0.23$, $p = 0.131$).

Table 1 shows the baseline aqueous humor levels of growth factors, growth factor receptors, inflammatory factors, and cytokines. The median baseline aqueous humor levels of sVEGFR-1, sVEGFR-2, VEGF, PlGF, PDGF-AA, sICAM-1, MCP-1, IL-6, IL-8, IL-12(p70), IL-13, and IP-10 were 362, 436, 53.3, 6.71, and 19.8 pg/mL, 16.4 ng/mL, and 1,558, 9.32, 11.4, 0.77, 0.55, and 133 pg/mL, respectively.

At baseline, there were significant correlations between the aqueous flare value and aqueous humor level of 6 factors/cytokines, i.e., sVEGFR-1, PlGF, MCP-1, sICAM-1, IL-6, and IP-10 (Table 2). However, there were no significant correlations between the aqueous flare value and aqueous humor level of the other factors/cytokines: sVEGFR-2, VEGF, PDGF-AA, IL-8, IL-12, and IL-13 (Table 2).

There was no significant correlation between the change in aqueous flare value and improvement in BCVA 1 month after anti-VEGF therapy ($\rho = -0.13$, $p = 0.393$; Fig. 3a). On the other hand, a significant correlation was noted between the change in aqueous flare value and improvement in CMT at 1 month ($\rho = 0.32$, $p = 0.028$; Fig. 3b).

Fig. 2. Aqueous flare value 1 month after intravitreal ranibizumab injection. The aqueous flare value decreased significantly from 16.3 ± 7.9 photon counts/ms at baseline to 12.4 ± 5.3 photon counts/ms 1 month after anti-VEGF therapy ($p < 0.001$).

Table 1. Aqueous humor levels of factors/cytokines at baseline

	sVEGFR-1, pg/mL	sVEGFR-2, pg/mL	VEGF, pg/mL	PIGF, pg/mL	PDGF-AA, pg/mL	sICAM-1, ng/mL	MCP-1, pg/mL	IL-6, pg/mL	IL-8, pg/mL	IL-12(p70), pg/mL	IL-13, pg/mL	IP-10 pg/mL
At baseline	362 (139–582)	436 (299–537)	53.3 (0.64–111)	6.71 (4.0–13.8)	19.8 (14.7–23.5)	16.4 (7.85–40.0)	1,558 (1,092–2,448)	9.32 (3.19–23.7)	11.4 (8.42–17.9)	0.77 (0.45–1.34)	0.55 (0.40–1.45)	133 (48.9–255)

Values express median (IQR). VEGF, vascular endothelial growth factor; sVEGFR, soluble VEGF receptor; PIGF, placental growth factor; PDGF, platelet-derived growth factor; sICAM, soluble intercellular adhesion molecule; MCP, monocyte chemoattractant protein; IL, interleukin.

Table 2. Correlations between aqueous humor factors/cytokines and aqueous flare values at baseline

	sVEGFR-1	sVEGFR-2	VEGF	PIGF	PDGF-AA	sICAM-1	MCP-1	IL-6	IL-8	IL-12(p70)	IL-13	IP-10
Aqueous flare at baseline												
<i>r</i>	0.30	0.17	0.01	0.47	0.26	0.47	0.29	0.32	0.20	–0.19	0.05	0.53
<i>p</i> value	0.043	0.256	0.873	<0.001	0.075	<0.001	0.049	0.032	0.170	0.195	0.741	<0.001

Spearman's rank-order correlation coefficient (*r*) was calculated. VEGF, vascular endothelial growth factor; sVEGFR, soluble VEGF receptor; PIGF, placental growth factor; PDGF, platelet-derived growth factor; sICAM, soluble intercellular adhesion molecule; MCP, monocyte chemoattractant protein; IL, interleukin.

Discussion

We identified a significant decrease in the aqueous flare value 1 month after initiation of anti-VEGF therapy for DME. This suggests that anti-VEGF therapy reduces inflammation in DME patients. Campochiaro et al. [10] recently proposed a positive feedback loop for VEGF in which vascular occlusion causes retinal ischemia and the release of VEGF by ischemic retinal cells, leading to the exacerbation of retinal nonperfusion by promoting leukostasis in association with the development of inflammation. Anti-VEGF therapy interrupts this positive feedback loop by neutralizing VEGF, so the aqueous flare value may have decreased in our DME patients due to the suppression of inflammation. We also demonstrated a significant positive correlation between the aqueous flare value and the aqueous humor levels of sVEGFR-1, a growth factor (PIGF), and several inflammatory factors/cytokines (sICAM-1, MCP-1, IL-6, and IP-10), indicating that ocular levels of these factors/cytokines are elevated in patients with higher flare values. Binding of VEGF and PIGF to VEGFR-1 induces leukocyte chemotaxis and promotes inflammation [11–13], while binding of VEGF to VEGFR-2 upregulates the expression of inflammatory factors such as MCP-1 and ICAM-1 via NF-κB [14–16]. Thus, both chemotaxis and leukocyte adhesion are promoted by VEGF and PIGF, leading to the development of inflammation. Accordingly, the aqueous flare value may be increased by the leakage of proteins from the iridal vessels after disruption of the blood-aqueous barrier by these factors. Anti-VEGF therapy would be expected to inhibit various inflammatory factors by suppressing signaling via VEGFR-1 and VEGFR-2, resulting in a decreased aqueous flare value.

In contrast to our findings, Morioka et al. [17] reported no significant change in the aqueous flare value 1 month after anti-VEGF therapy from that at baseline. However, their study population included patients who received retinal photocoagulation in the 6 months before enrollment, and/or intravitreal injection of antiangiogenic drugs or steroids in the 3 months before enrollment. Even after several months, retinal photocoagulation and intravitreal injection of antiangiogenic drugs or steroids could affect cytokine levels [18]; this may explain the difference between their results and ours.

In this study, we found a significant correlation between the change in aqueous flare value and the improvement in CMT 1 month after anti-VEGF therapy, suggesting that the CMT improvement might depend on the extent to which the inflammation is controlled. This notion

Fig. 3. Correlation between the change in aqueous flare value and BCVA or CMT 1 month after intravitreal ranibizumab injection. **a** There was no significant correlation between the change in aqueous flare value and the improvement in BCVA 1 month after anti-VEGF therapy ($\rho = -0.13$, $p = 0.393$). **b** A significant positive correlation was found between the change in aqueous flare value and improvement in CMT 1 month after anti-VEGF therapy ($\rho = 0.32$, $p = 0.028$).

is supported by a recent report that the mean decrease in CMT is significantly greater with combination therapy (ranibizumab + dexamethasone) than with ranibizumab alone [19], and a report that steroid-treated eyes require significantly fewer injections of anti-VEGF agents than control eyes [20]. As mentioned above, anti-VEGF therapy decreases the aqueous flare value and reduces vascular permeability by suppressing VEGF activity [10], explaining why the change in aqueous flare value was significantly correlated with the improvement in CMT 1 month after anti-VEGF therapy. However, there was no significant positive correlation between the change in aqueous flare value and improvement in BCVA 1 month after anti-VEGF therapy. This finding suggests that visual acuity did not improve along with the reduction in CMT. Indeed, the reduction in CMT observed in our study was not significantly correlated with an improvement in visual acuity. It is possible that recovery of visual acuity is a slower process and that there is a time lag between the improvement in BCVA and the changes in CMT. In support of this concept, it has been reported that the mean change in visual acuity was not significantly

greater with combination therapy compared to ranibizumab alone [19]. Therefore, an improvement in BCVA might not be directly related to the suppression of inflammation. Taken together with these reports, our findings suggest that the change in aqueous flare could be an index of the improvement in CMT in DME patients receiving anti-VEGF therapy for macular edema.

We also demonstrated a significant positive correlation between the aqueous flare value and aqueous humor levels of several factors. We recently reported that CMT shows a significant correlation with the aqueous humor levels of various factors in DME patients [6]; this may partially explain why the change in aqueous flare value was the only independent determinant of the improvement in CMT after anti-VEGF therapy. In contrast to our findings, Shiraya et al. [21] reported no correlation between changes in flare values and CMT in an IRI group. However, their study included patients who had received retinal photocoagulation and YAG capsulotomy in the 6 months before enrollment, and intravitreal injection of an antiangiogenic drug, or intravitreal or sub-Tenon injection of triamcinolone acetonide in the 3 months before

enrollment. Even after several months, these treatments can affect cytokine levels [18]. Shiraya et al. [21] also evaluated the correlation between the change rates in flare values and CMT 14 days after injection, unlike our evaluation which was carried out after 30 days. These differences between the study protocols might have contributed to the differences in results.

In conclusion, we identified a significant decrease in the aqueous flare value at 1 month after anti-VEGF therapy. Aqueous humor levels of inflammatory factors/cytokines were significantly correlated with the baseline aqueous flare values. In addition, there was a significant correlation between the change in aqueous flare value and the improvement in CMT 1 month after anti-VEGF therapy. These findings suggest that anti-VEGF therapy reduces intraocular subclinical inflammation, and that the aqueous flare value is influenced by multiple inflammatory factors/cytokines. In addition, the change in aqueous flare value may be an indicator of the improvement in CMT in patients receiving anti-VEGF therapy for DME.

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References

- 1 Antcliff RJ, Marshall J. The pathogenesis of edema in diabetic maculopathy. *Semin Ophthalmol*. 1999 Dec;14(4):223–32.
- 2 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 Oct;113(10):1706–12.
- 3 Nguyen QD, Tatlipinar S, Shah SM, Haller JA, Quinlan E, Sung J, et al. Vascular endothelial growth factor is a critical stimulus for diabetic macular edema. *Am J Ophthalmol*. 2006 Dec;142(6):961–9.
- 4 Wells JA, Glassman AR, Ayala AR, Jampol LM, Aiello LP, Antoszyk AN, et al.; Diabetic Retinopathy Clinical Research Network. Aflibercept, bevacizumab, or ranibizumab for diabetic macular edema. *N Engl J Med*. 2015 Mar;372(13):1193–203.
- 5 Noma H, Mimura T, Yasuda K, Shimura M. Role of inflammation in diabetic macular edema. *Ophthalmologica*. 2014;232(3):127–35.
- 6 Noma H, Mimura T, Yasuda K, Motohashi R, Kotake O, Shimura M. Aqueous Humor Levels of Soluble Vascular Endothelial Growth Factor Receptor and Inflammatory Factors in Diabetic Macular Edema. *Ophthalmologica*. 2017;238(1–2):81–8.
- 7 Elman MJ, Aiello LP, Beck RW, Bressler NM, Bressler SB, Edwards AR, et al. Randomized trial evaluating ranibizumab plus prompt or deferred laser or triamcinolone plus prompt laser for diabetic macular edema. *Ophthalmology*. 2010;117:1064–77.e35.
- 8 Mitchell P, Bandello F, Schmidt-Erfurth U, Lang GE, Massin P, Schlingemann RO, et al.; RESTORE study group. The RESTORE study: ranibizumab monotherapy or combined with laser versus laser monotherapy for diabetic macular edema. *Ophthalmology*. 2011 Apr;118(4):615–25.
- 9 Nguyen QD, Brown DM, Marcus DM, Boyer DS, Patel S, Feiner L, et al.; RISE and RIDE Research Group. Ranibizumab for diabetic macular edema: results from 2 phase III randomized trials: RISE and RIDE. *Ophthalmology*. 2012 Apr;119(4):789–801.
- 10 Campochiaro PA, Wyckoff CC, Shapiro H, Rubio RG, Ehrlich JS. Neutralization of vascular endothelial growth factor slows progression of retinal nonperfusion in patients with diabetic macular edema. *Ophthalmology*. 2014 Sep;121(9):1783–9.
- 11 Clauss M, Weich H, Breier G, Knies U, Röckl W, Waltenberger J, et al. The vascular endothelial growth factor receptor Flt-1 mediates biological activities. Implications for a functional role of placenta growth factor in monocyte activation and chemotaxis. *J Biol Chem*. 1996 Jul;271(30):17629–34.
- 12 Luttun A, Tjwa M, Moons L, Wu Y, Angelillo-Scherer A, Liao F, et al. Revascularization of ischemic tissues by PlGF treatment, and inhibition of tumor angiogenesis, arthritis and atherosclerosis by anti-Flt1. *Nat Med*. 2002 Aug;8(8):831–40.
- 13 Shibuya M. Vascular Endothelial Growth Factor (VEGF) and Its Receptor (VEGFR) Signaling in Angiogenesis: A Crucial Target for Anti- and Pro-Angiogenic Therapies. *Genes Cancer*. 2011 Dec;2(12):1097–105.
- 14 Ledebur HC, Parks TP. Transcriptional regulation of the intercellular adhesion molecule-1 gene by inflammatory cytokines in human endothelial cells. Essential roles of a variant NF-kappa B site and p65 homodimers. *J Biol Chem*. 1995 Jan;270(2):933–43.

Statement of Ethics

This study was conducted at the Department of Ophthalmology of Tokyo Medical University Hachioji Medical Center and approval was obtained from Institutional Review Board of Tokyo Medical University Hachioji Medical Center (IRB No. H-131). The procedures of the study conformed to the tenets of the Declaration of Helsinki and all patients gave written informed consent before enrollment.

Conflict of Interest Statement

No conflicting relationship exists for any author.

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Author Contributions

H.N. and M.S. were involved in the design and conduct of the study. Collection and management of the data were done by M.I., R.M., and H.N. Analysis and interpretation of the data were performed by K.Y. and M.S. Preparation of the first draft of the manuscript was done by M.I. and H.N. Review and approval of the manuscript were performed by H.G. and M.S. All authors read and approved the final manuscript.

- 15 Baldwin AS Jr. The NF-kappa B and I kappa B proteins: new discoveries and insights. [Annu Rev Immunol](#). 1996;14(1):649–83.
- 16 Marumo T, Schini-Kerth VB, Fisslthaler B, Busse R. Platelet-derived growth factor-stimulated superoxide anion production modulates activation of transcription factor NF-kappaB and expression of monocyte chemoattractant protein 1 in human aortic smooth muscle cells. [Circulation](#). 1997 Oct;96(7):2361–7.
- 17 Morioka M, Takamura Y, Yamada Y, Matsu-mura T, Gozawa M, Inatani M. Flare levels after intravitreal injection of ranibizumab, aflibercept, or triamcinolone acetonide for diabetic macular edema. [Graefes Arch Clin Exp Ophthalmol](#). 2018 Dec;256(12):2301–7.
- 18 Shimura M, Yasuda K, Nakazawa T, Abe T, Shiono T, Iida T, et al. Panretinal photocoagulation induces pro-inflammatory cytokines and macular thickening in high-risk proliferative diabetic retinopathy. [Graefes Arch Clin Exp Ophthalmol](#). 2009 Dec; 247(12):1617–24.
- 19 Maturi RK, Glassman AR, Liu D, Beck RW, Bhavsar AR, Bressler NM, et al.; Diabetic Retinopathy Clinical Research Network. Effect of Adding Dexamethasone to Continued Ranibizumab Treatment in Patients with Persistent Diabetic Macular Edema: A DRCR Network Phase 2 Randomized Clinical Trial. [JAMA Ophthalmol](#). 2018 Jan; 136(1):29–38.
- 20 Shimura M, Yasuda K, Minezaki T, Noma H. Reduction in the frequency of intravitreal bevacizumab administrations achieved by posterior subtenon injection of triamcinolone acetonide in patients with diffuse diabetic macular edema. [Jpn J Ophthalmol](#). 2016 Sep; 60(5):401–7.
- 21 Shiraya T, Kure K, Araki F, Kato S, Kaiya T. Correlation between anterior chamber flare changes and diabetic macular edema after intravitreal injection of ranibizumab and aflibercept. [Jpn J Ophthalmol](#). 2020 May; 64(3):250–6.