• Clinical Research •

Anti-VEGF reduces inflammatory features in macular edema secondary to retinal vein occlusion

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Abstract

● **AIM:** To investigate the anti-inflammatory effect of intravitreal injection of anti-vascular endothelial growth factor (anti-VEGF) in patients with macular edema secondary to retinal vein occlusion (RVO-ME).

● **METHODS:** Twenty-eight eyes from twenty-eight treatment-naïve patients (14 males and 14 females) with RVO-ME were included in this retrospective study. The retinal vein occlusion (RVO) was comprised of both central retinal vein occlusion (CRVO, *n*=14) and branch retinal vein occlusion (BRVO, *n*=14). Intravitreal injection of anti-VEGF reagents were administered monthly for three consecutive months, in which 18 patients were injected with ranibizumab and 10 patients were injected with conbercept. All eyes were imaged with optical coherence tomography angiography (OCTA) at baseline and 1wk after monthly intravitreal anti-VEGF injection. The visual acuity (VA), central macular thickness (CMT), the number of hyperreflective foci (HRF) recognized as an inflammatory sign in OCT images, and non-perfusion area (NPA), were compared before and after anti-VEGF treatments.

● **RESULTS:** The mean interval between baseline and follow-up was 29.4±0.79 (range, 27-48)d. Compared with the baseline, the VA improved (logMAR 1.5±0.1 *vs* 0.8±0.1, *P*<0.05) and CMT decreased (460±34.0 μm *vs* 268.8±12.0 μm, *P*<0.05), significantly, after anti-VEGF treatment. The number of HRF was decreased significantly (76.5±4.8 *vs* 47.8±4.3, *P*<0.05) after anti-VEGF treatment.

● **CONCLUSION:** Anti-VEGF therapy is effective in treating RVO-ME. The mechanisms for the decreased HRF and the reduction of NPA by anti-VEGF therapy merits further exploration.

● **KEYWORDS:** macular edema; retinal vein occlusion; anti-VEGF; hyperreflective foci; non-perfusion area

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INTRODUCTION

Retinal vein occlusion (RVO) is the second most common retinal vascular disease after diabetic retinopathy, resulting in visual impairment. The incidence of RVO is about 0.5%-1.8% in the general population[1-2]. The complications due to RVO include macular edema (RVO-ME), retinal

neovascularization with secondary vitreous hemorrhage, neovascular glaucoma, *etc*., which largely impaired the vision of patients. The pathogenesis of RVO-ME is multifactorial. The occluded and damaged blood vessels as well as retinal ischemia can result in local hypoxia with the increased hypoxia inducible factor-1 alpha (HIF-1α), resulting in elevated secretion of vascular endothelial growth factor (VEGF), which could cause vascular hyperpermeability and neovascularization[3-4]. Anti-VEGF treatment has been shown to be beneficial to patients with RVO-ME and becomes the first-line therapy in the treatment of RVO-ME[5-8]. Besides VEGF, other factors including inflammatory cells and cytokines were also associated with the pathogenesis of RVO-ME.

Increasing evidence suggested that hyperreflective foci (HRF) in retina were identified as the active inflammatory cells, especially microglia and macrophages, by using optical coherence tomography (OCT) or optical coherence tomography angiography (OCTA), indicating the inflammatory conditions in retina for patient with RVO-ME. HRF were first mentioned by Coscas *et al*[9] in patients with age-related macular degeneration (AMD) with spectral-domain OCT. Subsequently, HRF have been involved in many retinal diseases, such as RVO, diabetic retinopathy, choroideremia, and other retinal degenerative diseases[10-13]. Although its pathogenesis is still debated, HRF likely characterizes a progressive nature of an inflammatory retinal microenvironment.

During the clinical practice, we noticed that the RVO-ME patients with HRF benefit from anti-VEGF injections, demonstrating the improved visual acuity (VA), reduced central macular thickness (CMT) and HRF number, as well as the decreased non-perfusion area (NPA). Besides the direct anti-VEGF effect, we hypothesized that anti-VEGF reagents might exert anti-inflammatory effect in patients with RVO-ME. To address this question, we retrospectively reviewed 28 eyes from 28 treatment-naïve patients, who underwent three consecutive intravitreal injections of anti-VEGF reagents. The VA, CMT, the HRF number, and NPA size before and after intravitreal injections were quantified and compared.

SUBJECTS AND METHODS

Ethical Approval This study was approved by the Clinical Research Ethical Committee of Shanghai General Hospital affiliated to Shanghai Jiao Tong University (Permits No.2020KY205-2) and adhered to the principles of the Declaration of Helsinki. Informed consents were signed by all the participants.

Patients The present study was a retrospective cohort study, including 28 treatment-naïve patients, aged 64.2±2.1 years old. The patients were comprised of 14 males (50%) and 14 females (50%). The RVO included 14 BRVO and 14 CRVO. This retrospective study was conducted in the Department of Ophthalmology, Shanghai General Hospital affiliated to Shanghai Jiao Tong University School of Medicine, Shanghai, China between August 26, 2019, and July 30, 2020. Participants who received intravitreal injections of anti-VEGF drugs for three months were included in the study. The eyes with any co-existing ocular diseases, including diabetic retinopathy, hypertensive retinopathy, AMD, or uveitis, *etc*., were excluded.

At the initial examination, comprehensive ophthalmic examinations were performed for every patient, including OCTA, fundus photography, best-corrected visual acuity (BCVA), intraocular pressure and anterior segment evaluation using slit-lamp biomicroscopy. Follow-up examinations were conducted 1wk after each intravitreal injection.

Intravitreal Injection of Anti-VEGF Reagents The intravitreal injection was conducted at the temporal limbus through the eyeball's pars plana under aseptic conditions in the operating room. Twenty-eight patients received three consecutive intravitreal injections of ranibizumab at the concentration of 0.5 mg/ 0.05 mL (Novartis Pharma Stein AG, Switzerland, n=18) or conbercept at the concentration of 0.5 mg/ 0.05 mL (Chengdu Kang Hong Biotech Co., Ltd., Sichuan Province, China, n=10) with a 30-gauge needle. Each injection interval allowed a variation of 1wk. The participants were treated with three monthly intravitreal injections until the macular edema (ME) was resolved.

Optical Coherence Tomography Angiography Examination Retinal microvasculature was visualized by using the RTVue XR Avanti OCT system (Optovue, Inc., Fremont, CA, USA), and the quantification was carried out using the manufacturer's AngioVue software. The scanning was centered on the fovea with an area of 6×6 mm^2.

CMT measured with OCTA was calculated as the average retinal thickness in a 1-mm-diameter circular region centered at the fovea which was automatically analyzed by OCTA.

The HRF number was manually counted in the whole retina within a 6-mm diameter centered on the fovea using a fovea-spanning horizontal B-scan. HRF in OCTA was defined as a discrete and well-circumscribed dot-shaped lesion of equal or higher reflectivity than the retinal pigment epithelium (PRE) band. The maximal diameter of HRF was limited within the 20 to 50 μm range in order to exclude small counting noise signals (less than 20 μm) and prevent large hyperreflective clumps, such as hard exudates. Poor-quality images with a signal strength index less than 4/10 were excluded. The quantification of HRF was conducted independently by two experienced physicians.

The NPA was outlined manually in enface image of the superficial capillary plexus (SCP) with 6×6 mm^2 scanning

area in OCTA and analyzed automatically with the OCTA auto-segmentation software. The SCP was segmented as 3 μm below the internal limiting membrane and 15 μm below the inner plexiform layer.

Statistical Analysis The data were analyzed by using the IBM SPSS Statistics 21 software. All values are presented as a number or mean±standard deviation. The VA was expressed as the logarithm of the minimum angle of resolution (logMAR). A paired *t*-test was employed to compare BCVA, the number of HRF, and NPA between the baseline and after 3 consecutive monthly anti-VEGF injections. A *P*-value less than 0.05 was determined as statistically significant difference.

RESULTS

Patient Information The baseline clinical features of 28 eyes were shown in Table 1. The participants are comprised of 14 females (50%) and 14 males (50%). The mean age of patients was 64.2±2.1 years old, ranging from 50 to 78 years old, with 63.2±2.5 years old for BRVO and 64.8±3.4 years old for CRVO. The RVO included both BRVO (50%, *n*=14) and CRVO (50%, *n*=14). All participants underwent three consecutive monthly injections of ranibizumab (18 patients) or conbercept (10 patients). Eight patients with BRVO and 10 patients with CRVO were injected with ranibizumab; and 6 patients with BRVO and 4 patients with CRVO were injected with conbercept. The mean interval between baseline and final follow-up was 108.1±8.7 (range 56-240)d.

Visual Acuity Significantly Improved after Anti-VEGF Treatment BCVA improved significantly from baseline to the final follow-up, and the mean change of BCVA was -0.8±0.1 for RVO group (Table 1). Figure 1 demonstrated the changes of VA before and after the treatment. After three consecutive injections of anti-VEGF reagents, the VA significantly increased in all three groups, RVO (1.5±0.1 *vs* 0.8±0.1, *n*=28, *P*<0.05), BRVO (1.4±0.2 *vs* 0.6±0.1, *n*=14, *P*<0.05), and CRVO (1.6±0.1 *vs* 1.0±0.2, *n*=14, *P*<0.05).

To observe the efficacy of two different anti-VEGF reagents, we sub-grouped the patients and analyzed the effect based on ranibizumab and conbercept injections. In Table 2, for ranibizumab treatment, the VA increased in RVO (1.4±0.1 *vs* 0.6±0.1, *n*=18, *P*<0.05), BRVO (1.2±0.2 *vs* 0.3±0.1, *n*=8, *P*<0.05), and CRVO (1.6±0.2 *vs* 0.9±0.2, *n*=10, *P*<0.05); and for conbercept treatment, the VA was increased in RVO (1.7±0.2 *vs* 1.0±0.1, *n*=10, *P*<0.05), BRVO (1.7±0.3 *vs* 0.8±0.2, *n*=6, *P*<0.05), and CRVO (1.7±0.1 *vs* 1.2±0.2, *n*=4, *P*<0.05). No significant difference has been found in term of VA improvement for each sub-group between ranibizumab and conbercept treatment.

Central Macular Thickness Significantly Decreased After Intravitreal Injection CMT is a sensitive parameter to evaluate RVO-ME. In our study, the CMT reduced significantly after anti-VEGF injections (Table 1 and Figure 2), RVO (460±34.0 *vs* 268.8±12.0 μm, *n*=28, *P*<0.05), BRVO (413±47 *vs* 255±11 μm, *n*=14, *P*<0.05), and CRVO (512±47 *vs* 283±22 μm, *n*=14, *P*<0.05).

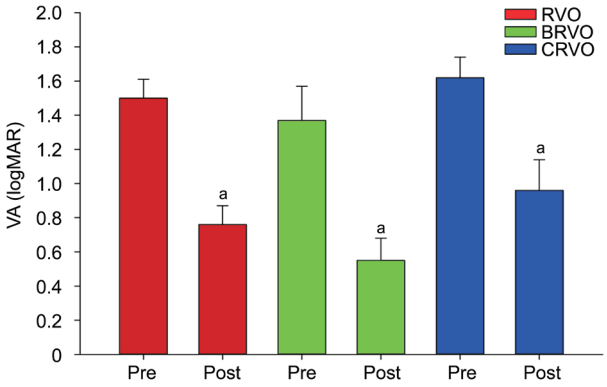

Figure 1 The changes in VA before and after treatment in patients with RVO The mean VA was significantly increased after treatment (*n*=28, [a]*P*<0.05, paired *t*-test). Pre: Pre-treatment; Post: Post-treatment after 3 consecutive injections. RVO: Combined BRVO and CRVO. RVO: Retinal vein occlusion; VA: Visual acuity; BRVO: Branch retinal vein occlusion; CRVO: Central retinal vein occlusion.

Table 1 Baseline characteristics of patients with RVO and comparation of VA, CMT, NPA, and HRF between baseline and after the intravitreal anti-VEGF treatment

Items	Baseline	Treatment	*P*
Eyes (*n*)	28	-	-
Sex (male/female)	14/14	-	-
Age (y)	64.2±2.1	-	-
BRVO (*n*)	14	-	-
CRVO (*n*)	14	-	-
Ranibizumab (*n*)	18	-	-
Conbercept (*n*)	10	-	-
BCVA (logMAR)-BRVO	1.4±0.2	0.6±0.1	<0.05[a]
BCVA (logMAR)-CRVO	1.6±0.1	1.0±0.2	<0.05[a]
BCVA (logMAR)-RVO	1.5±0.1	0.8±0.1	<0.05[a]
CMT-BRVO (μm)	413±47	255±11	<0.05[a]
CMT-CRVO (μm)	512±48	283±22	<0.05[a]
CMT-RVO (μm)	460±34	268.8±12	<0.05[a]
HRF-BRVO (*n*)	68.1±5.6	40.6±4.7	<0.05[a]
HRF-CRVO (*n*)	84.9±7.3	55±6.9	<0.05[a]
HRF-RVO (*n*)	76.5±4.8	47.8±4.3	<0.05[a]
NPA-BRVO (mm^2)	7.2±1.5	6.8±1.3	0.85
NPA-CRVO (mm^2)	10.4±1.4	7.9±1.4	0.27
NPA-RVO (mm^2)	8.9±1.0	7.4±1.0	0.32

[a]*P*<0.05. RVO: Retinal vein occlusion; VA: Visual acuity; CMT: Central macular thickness; NPA: Non-perfusion area; HRF: Hyperreflective foci; VEGF: Vascular endothelial growth factor; BRVO: Branch retinal vein occlusion; CRVO: Central retinal vein occlusion; BCVA: Best-corrected visual acuity.

Table 2 The changes of VA, CMT, HRF and NPA after ranibizumab or conbercept treatments

Parameters	Ranibizumab	Conbercept	P^a	P^b
Age (y)	63.2±2.6	65.3±3.6	-	-
Sex (male/female)	12/6	2/8	-	-
Eyes	18	10	-	-
BRVO (*n*)	8	6	-	-
CRVO (*n*)	10	4	-	-
BCVA-BRVO (logMAR)				
Baseline	1.2±0.2	1.7±0.3	-	-
Treatment	0.3±0.1	0.8±0.2	<0.05/<0.05	-
Difference	0.8±0.2	0.9±0.2	-	>0.05
BCVA-CRVO (logMAR)				
Baseline	1.6±0.2	1.7±0.1	-	-
Treatment	0.9±0.2	1.2±0.2	<0.05/<0.05	-
Difference	0.7±0.2	0.6±0.1	-	>0.05
BCVA-RVO (logMAR)				
Baseline	1.4±0.1	1.7±0.2	-	-
Treatment	0.6±0.1	1.0±0.1	<0.05/<0.05	-
Difference	0.8±0.1	0.75±0.1	-	>0.05
CMT-BRVO (μm)				
Baseline	405±48	419±89	-	-
Treatment	276±13	229±11	>0.05/>0.05	-
Difference	87±36	228±97	-	>0.05
CMT-CRVO (μm)				
Baseline	578±46	358±83	-	-
Treatment	290±29	264±26	<0.05/>0.05	-
Difference	270±53	70±52	-	>0.05
CMT-RVO (μm)				
Baseline	506±38	399±61	-	-
Treatment	284±17	242±12	<0.05/<0.05	-
Difference	184±39	165±64	-	>0.05
HRF-BRVO (*n*)				
Baseline	74.2±9.6	67.4±4.6	-	-
Treatment	43.0±8.2	35.6±2.0	>0.05/>0.05	-
Difference	30.2±13.1	31.6±13.5	-	>0.05
HRF-CRVO (*n*)				
Baseline	90.7±9.3	60.1±10.1	-	-
Treatment	66.7±6.7	27.7±3.4	<0.05/>0.05	-
Difference	33.4±13.1	14.9±7.8	-	>0.05
HRF-RVO (*n*)				
Baseline	83.8±6.7	64.9±4.5	-	-
Treatment	55.8±5.8	33.0±2.2	<0.05/<0.05	-
Difference	26.2±6.7	28.8±8.3	-	>0.05
NPA-BRVO (mm^2)				
Baseline	5.1±0.8	10.3±2.5	-	-
Treatment	4.7±0.8	8.4±2.2	>0.05/>0.05	-
Difference	1.2±0.4	1.8±0.8	-	>0.05
NPA-CRVO (mm^2)				
Baseline	10.4±1.7	11.6±2.9	-	-
Treatment	7.9±1.8	7.9±2.9	>0.05/>0.05	-
Difference	3.1±0.9	3.8±1.2	-	>0.05
NPA-RVO (mm^2)				
Baseline	7.6±4.6	10.9±1.7	-	-
Treatment	6.8±4.6	8.2±1.6	>0.05/>0.05	-
Difference	3.1±1.2	2.7±0.7	-	>0.05

[a]The comparison between baseline and treatment (ranibizumab/conbercept); [b]The comparison between the differences (baseline-treatment) of ranibizumab and conbercept treatment groups. RVO: Retinal vein occlusion; VA: Visual acuity; CMT: Central macular thickness; NPA: Non-perfusion area; HRF: Hyperreflective foci; VEGF: Vascular endothelial growth factor; BRVO: Branch retinal vein occlusion; CRVO: Central retinal vein occlusion; BCVA: Best-corrected visual acuity.

For ranibizumab treatment group, the CMT decreased in RVO (506±38 *vs* 284±17 μm, n=18, P<0.05), and CRVO (578±46 *vs* 290±29 μm, n=10, P<0.05; Table 2). As for conbercept treatment groups, the CMT decreased in RVO (399±61 *vs* 242±12 μm, n=10, P<0.05; Table 2). No obvious difference for the reduction of CMT was observed for each sub-group between ranibizumab and conbercept treatment.

Number of HRF significantly decreased by anti-VEGF HRF was identified as active macrophages and/or microglia in retina on OCT or OCTA examination. In this study, HRF was shown to be distributed throughout the whole retina, especially in the inner retina. HRF number significantly reduced in RVO (76.5±4.8 *vs* 47.8±4.3, n=28, P<0.05), BRVO (68.1±5.6 *vs* 40.6±4.7, n=14, P<0.05), and CRVO (84.9±7.3 *vs* 55±6.9, n=14, P<0.05), respectively, following anti-VEGF treatment (Table 1 and Figure 3).

For ranibizumab treatment, the number of HRF decreased in RVO (83.8±6.7 *vs* 55.8±5.8, n=18, P<0.05), and CRVO (90.7±9.3 *vs* 66.7±6.7, n=10, P<0.05; Table 2). As for conbercept treatment group, the number of HRF decreased in RVO (64.9±4.5 *vs* 33.0±2.2, n=10, P<0.05; Table 2). No significant difference for HRF reduction was detected among three groups between ranibizumab and conbercept treatments.

Size of NPA Gradually Decreased After Three Consecutive Anti-VEGF Injections NPA reflected the non-perfusion of retinal capillaries due to transient occlusion of leukocyte in retinal blood vessels or permanent dropout of retinal capillaries forming acellular capillaries. To see whether or not anti-VEGF reagent could reduce NPA, we compared the NPA before and after three consecutive injections. As shown in Table 1, there was slightly increase of NPA for CRVO (10.4±1.4 mm^2) than BRVO (8.9±1.0 mm^2), but no significant difference, in the defined macular region (6×6 mm^2) at baseline. After anti-VEGF treatment, we observed gradual reduction of NPA after three consecutive injections in patients with RVO, 8.9±1.0 mm^2 (baseline), 8.5±1.0 mm^2 (after 1st injection), 8.1±1.0 mm^2 (after 2nd injection), and 7.4±1.0 mm^2 (after 3rd injection), although no significant difference was observed (Figure 4).

To see the effect of different anti-VEGF reagents on NPA, we analyzed and compared their effect on the changes of NPA. The data showed that, in ranibizumab and conbercept treatment group, NPA decreased in RVO, BRVO, and CRVO (Table 2) but with no significant difference. No obvious change was shown in NPA reduction between ranibizumab and conbercept treatments.

DISCUSSION

With an estimated 16 million patients worldwide, RVO become one of the most common retinal vascular diseases in adults[14-15]. In our study, the VA improved and the CMT decreased significantly in patients with RVO-ME after anti-VEGF treatments. The HRF also decreased significantly, accompanied with progressive reduction of NPA after three consecutive anti-VEGF treatments. This study indicated that retinal inflammation might play a contributory role in the pathogenesis of RVO-ME.

The pathogenesis of RVO-ME is complicated, in which ischemia and hypoxia plays essential roles in the formation of RVO-ME. Ischemia and hypoxia could stabilize HIF-1α and leads to elevated secretion of VEGF and other down-stream targets. In human, VEGF family contains five members, including VEGF-A (usually named as VEGF), VEGF-B, VEGF-C, VEGF-D, as well as placental growth factor (PGF). Both VEGF and PGF play important roles in the formation of ME through inducing the breakdown of blood-retinal barrier (BRB). Besides, the inflammation is considered as a key player in RVO. Previous studies reported that several inflammatory factors, other than VEGF and PGF, contributed to the pathogenesis of RVO-ME, including angiotensin II, interleukin (IL)-1β, IL-6, IL-8, basic fibroblast growth factor (bFGF), monocyte chemoattractant protein 1 (MCP-1), and PGF, *etc*[16-18]. Through binding VEGF receptors (VEGFR) on endothelial cells, VEGF and PGF induced the up-regulation of intercellular cell adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1) on endothelial cells, enhancing the leukocyte adhesion to the vessel wall, thus leading to leukostasis, retinal non-perfusion and BRB breakdown. Chronic inflammation will result in acellular capillaries, aggregating the retinal hypoxia. VEGF and PGF also facilitate the proliferation and activation of microglia and macrophage through VEGFR. The HRF on OCT or OCTA mainly refers to the inflammatory cells in retina, such as microglia and macrophages. These inflammatory cells, including leukocytes, will release pro-inflammatory factors, causing BRB breakdown, ME, neuronal damage, and visual deterioration[19-22].

We hypothesized that anti-VEGF reagents, by antagonizing VEGF and/or PGF, blocked the activation of VEGFR both on endothelial cells and inflammatory cells (microglia, macrophage, and leukocyte, *etc*.), thus down-regulated adhesion molecules of endothelial cell and deactivated the inflammatory cells as well as the inflammatory factor release. In this way, the leukostasis was alleviated and NPA was improved, and the HRF also decreased by anti-VEGF treatment, indicating an anti-inflammatory effect of anti-VEGF reagents.

Although no statistically significant difference was found, we observed the amelioration of NPA in patients with RVO after anti-VEGF treatment (Figure 4). The improved NPA might be due to transient adhesion of leukocyte to endothelial cells *via* CD11b/ICAM-1 interaction, and anti-VEGF treatment disrupted the interaction between leukocyte

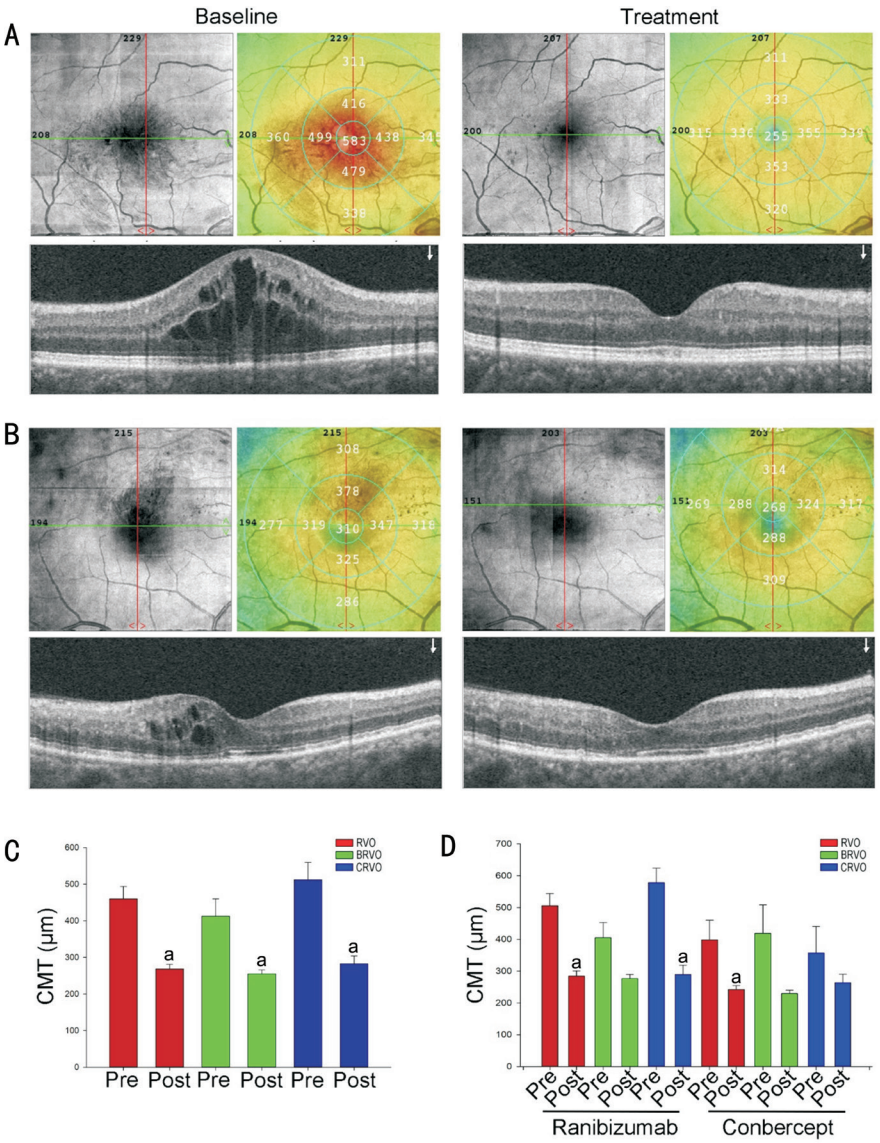

Figure 2 The macular edema was decreased significantly after anti-VEGF treatments A: Representative images of a 49 years old female patient with CRVO-ME at baseline and after three monthly intravitreal ranibizumab injections (Treatment). B: Representative images of a 62 years old female patient with BRVO-ME at baseline and after three monthly intravitreal conbercept injections. C: Sub-group quantitation of the changes of CMT in patients before and after treatment. D: The changes of CMT in patients based on anti-VEGF reagents. n=28, ^aP<0.05. Pre: Pre-operation; Post: Post-operation after 3 consecutive injections. RVO: Combined BRVO and CRVO. CMT: Central macular thickness; BRVO: Branch retinal vein occlusion; CRVO: Central retinal vein occlusion; BRVO-ME: Branch retinal vein occlusion include macular edema; CRVO-ME: Central retinal vein occlusion include macular edema; VEGF: Vascular endothelial growth factor.

and endothelial cells and re-opened the occluded capillaries. Thus we found the gradual improvement of NPA in patients with RVO after anti-VEGF therapy. But for some patients, the initial NPA cannot be identified easily due to massive, intensive hemorrhage obscured the direct observation of non-perfusion in retina. For some NPA caused by capillary dropout, it can be extrapolated that this non-perfusion cannot be alleviated due to the permanent capillary obliteration because anti-VEGF treatment cannot re-vascularize the NPA in a timely manner. So, the patients with RVO are suggested to initiate anti-VEGF treatment as soon as possible to decrease and eliminate the non-perfusion caused by early transient occlusion by leukocytes, and thus avoid permanent capillary dropout (acellular capillaries).

There are still some shortcomings in the current research. First, the sample size of this clinical study was comparatively small, that may affect the comparisons. Second, the study was a short-term observation, which needs a long-term follow-up. Last, OCTA requires consistent cooperation from the patients, and small vibration might make it difficult to perform the comparison among different groups. Therefore, long-term evaluation of the efficacy of anti-VEGF reagents and large sample multi-center studies are needed in the treatment of RVO-ME. Besides OCTA, other multimodal ophthalmic

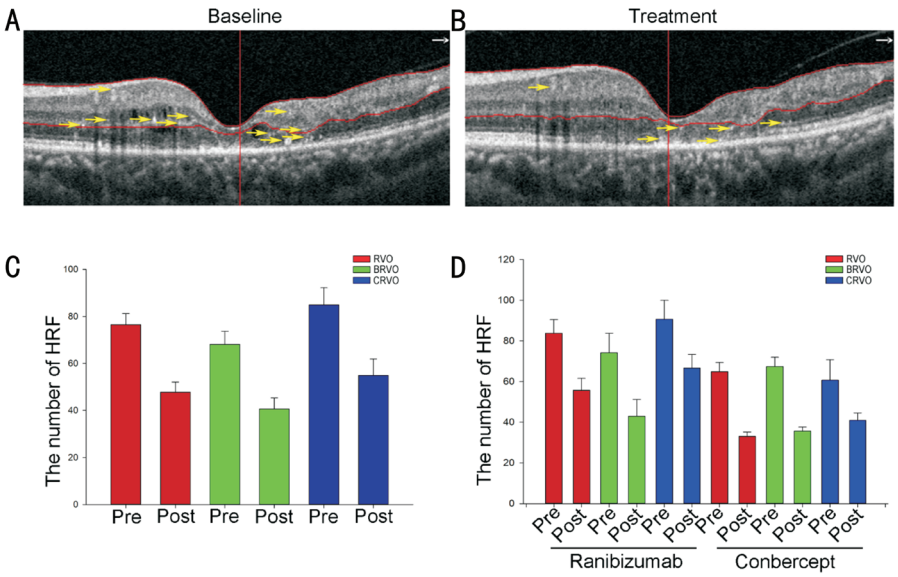

Figure 3 The changes of HRF before and after anti-VEGF treatment Representative images of one patient with BRVO-ME showing the HRF at baseline (A) and after three monthly intravitreal anti-VEGF injections (B). The yellow arrow indicates the HRF. C: Quantitation of HRF in patients before and after treatment. *n*=28. D: The changes of the numbers about HRF in patients based on anti-VEGF reagents. *n*=28. HRF: Hyperreflective foci; VEGF: Vascular endothelial growth factor; BRVO-ME: Branch retinal vein occlusion include macular edema.

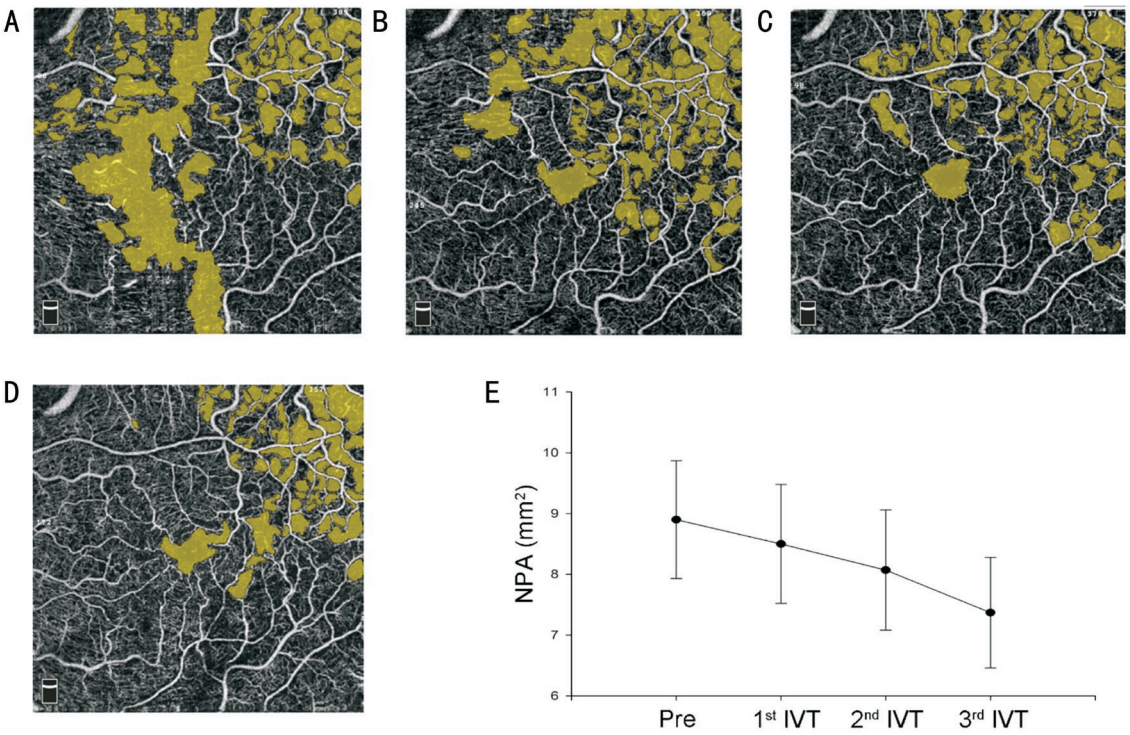

Figure 4 Representative image showing the gradual decrease of NPA before and after treatment OCTA examination showed a 67-year old patient with CRVO-ME at the baseline (A), and after 1st (B), 2nd (C), and 3rd intravitreal injections (D). The NPA was outlined in yellow. E: The quantitation of NPA at baseline and after 1 to 3 intravitreal injections (*n*=28). The mean size of NFA was decreased after treatment, but there was no significant difference between before and after treatments (*n*=28, paired *t*-test). OCTA: Optical coherence tomography angiography; CRVO-ME: Central retinal vein occlusion include macular edema; NPA: Non-perfusion area; IVT: Intravitreal injections.

imagings are required for evaluation of RVO-ME before and after the treatment.

In summary, retinal inflammation plays a critical role in RVO-ME. As detected with OCTA, besides macular edema, HRF and NPA were also observed. In the pathogenesis of RVO-ME, the retina was stressed under ischemia and hypoxia, which stabilized HIF-1α and increased the production of its down-stream targets, including VEGF, PGF, and VEGFR. Through binding VEGFR, VEGF/PGF enhanced the expression of adhesion molecules on endothelial cells, such as

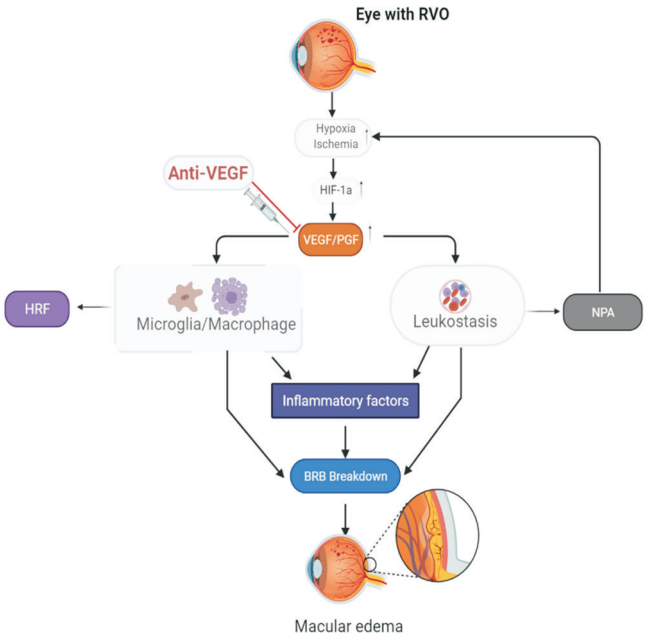

Figure 5 The schematic diagram showing the possible mechanisms of macular edema secondary to RVO and the anti-inflammatory effect of anti-VEGF treatment to reduce inflammation by breaking down the vicious cycle in patients with RVO RVO: Retinal vein occlusion; VEGF: Vascular endothelial growth factor.

ICAM-1 and VCAM-1, which promoted leukostasis, leading to increased NPA[16,23-25] and aggregating the retinal hypoxia. VEGF/PGF also promoted the activation of inflammatory cells *via* activating VEGFR on above cells, which were observed as increased number of HRF on OCTA. The activated inflammatory cells, such as macrophage and microglia, increased production of the inflammatory factors, such as IL-1β and IL-6, and MCP-1, further aggravating BRB breakdown and macular edema. The increasing NPA and activation of inflammation constitute a vicious cycle in the pathogenesis of RVO-ME. Anti-VEGF treatments, by antagonizing VEGF and/or PGF, breakdown the vicious cycle and ameliorate the inflammation and retinal hypoxia, as proposed in Figure 5. However, the specific mechanisms for anti-VEGF therapy in the reduction of HRF and NPA warrant further investigation to fully elucidate the anti-inflammatory effect of anti-VEGF in RVO-ME treatment.

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