

Effect of anti-VEGF treatment on nonperfusion areas in ischemic retinopathy

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Abstract

• In recent years, retinal ischemia such as that which occurs in diabetic retinopathy (DR) and retinal vein occlusion (RVO) has become a hotspot of ischemic retinopathy research. High levels of vascular endothelial growth factor (VEGF) are recognized as a major cause of macular edema (ME) in DR and RVO. High concentrations of VEGF in the vitreous can lead to serious retinal ischemia and hypoxia and form retinal nonperfusion areas (NPAs). Different levels of retinal ischemia can represent disease severity and progression. Anti-VEGF therapy as the first-line treatment for ME has been found to be effective in improving vision, but there are still disputes about whether anti-VEGF therapy could improve retinal ischemia and achieve reperfusion of previously developed retinal NPAs. Here, we review and summarize studies of the effects of anti-VEGF drugs on retinal ischemia, especially NPAs.

• **KEYWORDS:** anti-vascular endothelial growth factor; retinal nonperfusion areas; ischemic retinopathy; macular ischemia

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INTRODUCTION

Diabetic retinopathy (DR) and retinal vein occlusion (RVO) are the two most common ischemic retinopathies in the world. Retinal ischemia is usually caused by retinal

vascular diseases and is characterized by decreased retinal blood perfusion and the appearance of retinal nonperfusion areas (NPAs), and NPAs are considered to be a major cause of vision loss in ischemic retinopathies. The ischemic retina produces vascular endothelial growth factor (VEGF). High concentrations of VEGF in the vitreous can further aggravate retinal ischemia and hypoxia, usually lead to retinal edema to cause vision loss. RVO consists of central retinal vein occlusion (CRVO) and branch retinal vein occlusion (BRVO), and the occlusion of these veins leads to macular edema (ME), hemorrhage, appearance of NPAs, and even cause anterior segment neovascularization especially in CRVO, which is similar to that found in DR^[1]. When the macular region is affected by retinal ischemia, patients present with varying degrees of macular ischemia (MI). According to the Early Treatment Diabetic Retinopathy Study (ETDRS) Report Number 11, MI is defined as foveal avascular zone (FAZ) area expansion, disappearance of part of the macular arch ring capillary network, retinal capillary loss and the appearance of macular nonperfusion (MNP) areas^[2]. Anti-vascular endothelial growth factor (anti-VEGF) therapy has been recommended as the first-line treatment for DR and RVO, which improves retinal ischemia and hypoxia by blocking the pathway of VEGF^[3-4].

FLUORESCEIN ANGIOGRAPHY AND OPTICAL COHERENCE TOMOGRAPHY ANGIOGRAPHY FOR THE DETECTION OF NPAS

Fluorescein angiography (FA) is still considered the gold standard for detecting retinal vasculature diseases. The ETDRS group includes FA among the techniques for assessing retinal ischemic damage, and this technique enables the staging of disease severity and visualization of microaneurysms, venous beading, retinal ischemia and NPAs using seven standard fields that capture the central posterior 90° of the retina^[5]. FA provides some flow information, such as an estimation of blood velocity and assessment of vascular leakage. The intraretinal capillary occlusion or dropout characteristic of retinal NPAs is visualized by FA as a dark area between the relatively large retinal vessels^[6].

However, FA is an invasive test requiring contrast injection that cannot be easily repeated and is not suitable for patients with severe renal insufficiency or allergies to contrast media.

In recent years, optical coherence tomography angiography (OCTA) technology has been rapidly developed in the field of ophthalmology for the visualization of microaneurysms and retinal NPAs. OCTA enables a closer observation of each layer of retinal capillaries. The ability of OCTA to noninvasively visualize the retinal vasculature makes it a better modality for disease detection and monitoring of MI in patients with DR and RVO. OCTA can provide structural and quantifiable images of the FAZ and the macular capillaries at different retinal plexuses. The superficial plexus is an interconnected capillary plexus connected by arterioles and venules, and the deep capillary plexus is organized in capillary vortexes, whereas the deep plexus corresponds to the macular photoreceptors and is important for maintaining photoreceptor and outer retinal oxygen supplies^[7-8]. The choriocapillaris and retinal capillary perfusion density are reduced in DR compared with healthy eyes, while the FAZ area and perimeter are increased^[9]. Some researchers have found that in the eyes of patients with diabetes with ME, vessel density values in the deep retinal plexus were significantly lower than in the eyes of patients with diabetes without ME; however, it was not determined whether the deep vessel density changes were due to edema or risk factors for edema, such as physical displacement of retinal capillaries or the decorrelation signal of OCTA^[10].

Many studies have proven that OCTA is highly sensitive and consistent compared with FA, and OCTA could be considered a valid and reliable method to evaluate pathological ischemic retinal microvascular changes^[11]. Couturier *et al*^[12] found that the detection rate of NPAs was higher with OCTA than with FA. Spaide *et al*^[13] found that OCTA images of MI were sharper than those obtained *via* FA because the masking effect caused by leakage in FA does not affect the OCTA images. OCTA may be clinically useful to evaluate the microvascular status and therapeutic effect of treatments. OCTA acquires high-resolution images of macular areas by capturing the signals of moving red blood cells; however, OCTA does not detect retinal capillary blood flow less than 0.3 mm per second and cannot detect blood flow in microaneurysms^[14].

Another limitation of OCTA is its small field of view. However, the rapid development of imaging technology has provided an option for wider-field FA and OCTA. Ultra-widfield (UWF) FA and widefield (WF) OCTA offer great advantages for the detection of NPAs in retinal vascular diseases, including DR and RVO^[15]. UWF-FA has emerged as a powerful tool for quantifying areas of NPAs, particularly the peripheral retina, and identifying patients at risk for progression to proliferative retinopathy^[16]. Some researchers have found that peripheral NPAs statistically correlate with central NPAs in patients with severe non proliferative diabetic retinopathy (NPDR) and proliferative diabetic retinopathy (PDR), and patients with

more advanced retinopathy have more NPAs^[17]. Additionally, peripheral NPAs could be a strong predictor of central and MI^[18].

FA and OCTA has lots of advantages in observing the retinal ischemia and MI. Areas of retinal NPAs and ISI obtained by FA and UWF-FA represent the severity of peripheral retinal ischemia. While FAZ related indicators and macular vessel density calculated by OCTA represent the macular perfusion status. These ischemic indicators can be used as biomarkers to observe the severity of diseases and therapeutic effects.

EFFECT OF NPAS ON VISUAL ACUITY AND MACULAR EDEMA

The appearance of peripheral retinal NPAs and MNPs usually indicates worse visual acuity (VA). Antaki *et al*^[17] demonstrated a more direct correlation between the proportion of retinal NPAs in the peripheral area and VA. The larger the peripheral component of NPAs, the worse the VA in patients with PDR. Samara *et al*^[19] confirmed decreased macular vascular density in both the superficial and deep vascular networks, and vascular density and FAZ area appeared to correlate with visual function by using quantitative OCTA measurements. AttaAllah *et al*^[20] found that VA was significantly correlated with FAZ area and macular vessel density at the superficial retinal plexus in the eyes of patients with diabetes with ME. Compared with RVO patients with fewer NPAs, patients with a peripheral retinal ischemic index >10% had more severe ME and worse VA at baseline but gained a greater decrease in mean foveal central thickness and better improvement in VA after treatment^[21]. The WAVE study, focused on the relationship between the ischemic index in different retinal regions and ME, revealed found that the severity of ME was correlated with the ischemic index of the entire retinal area and perimacular area^[22]. Different degrees of peripheral retinal NPAs were correlated with the severity of disease, and an area of NPAs >23% of the total visible retina was likely associated with neovascularization in DR^[16]. Nicholson *et al*^[23] observed that the difference in NPA was more pronounced in the periphery than in the posterior pole, and patients with eyes with at least 107.3 disc areas of NPAs were at risk for PDR.

ANTI-VEGF TREATMENT

Anti-VEGF treatment has been proven to be effective in improving retinal edema and vision in DR and RVO. However, retinal perfusion status at regular intervals has only been evaluated in a small number of multicenter clinical trials, even though DR and RVO are known ischemic retinopathies. To date, whether anti-VEGF therapy could aggravate retinal ischemia remains controversial. In the past decade, some clinical studies have suggested that blocking VEGF might be harmful to retinal vascular integrity, especially in patients with preexisting retinal ischemia; anti-VEGF therapy aggravated

retinal ischemia in these patients because the NPAs enlarged after anti-VEGF therapy^[24]. Some researchers have speculated that anti-VEGF treatment may inhibit the normal physiological concentration of VEGF, leading to the aggravation of retinal ischemia^[25]. However, an increasing number of studies have revealed that retinal ischemia does not worsen after anti-VEGF therapy^[26-27]. Whether treatment with anti-VEGF drugs can worsen retinal perfusion status and macular perfusion status has been controversial, especially when considerable NPAs are present at baseline.

EFFECT OF ANTI-VEGF TREATMENTS ON NPAS IN DR AND RVO

Retinal ischemia has been of great concern in clinical studies, and the appearance of NPAs or increase of existing NPAs are major signs of the aggravation of ischemic retinopathy. NPAs caused by RVO can involve one quadrant or multiple quadrants^[28]. Many early studies suggested that retinal ischemia would develop gradually and irreversibly, irrespective of anti-VEGF treatment. In 2011, Terui *et al*^[29] observed the deterioration of retinal perfusion status in the eyes of patients with BRVO after bevacizumab treatment. They also found that new retinal NPAs developed after intravitreal bevacizumab treatment in 3 of 37 eyes that had no nonperfusion at baseline, and the incidence of a significant increase in NPAs greater than 1.0 in the disc area was 1.7%. A rabbit retinal neovascularization model proved that a sudden decrease in VEGF concentration may cause normal capillaries to close^[30]. Therefore, some researchers believe that anti-VEGF treatment may aggravate retinal ischemia; however, it is not yet clear whether the incidence of severe ischemic changes after anti-VEGF therapy is truly because of the blockage of VEGF or if the findings instead result from spontaneous changes during the natural course of disease^[31].

To date, there have been disputes about whether anti-VEGF therapy could aggravate retinal ischemia. However, an increasing number of studies have shown that anti-VEGF treatment does not aggravate retinal ischemia, and long-term treatment with anti-VEGF treatment can improve DR severity and prevent further worsening, in particular in mild and severe DR^[32]. Some researchers have found that anti-VEGF treatment can reduce the progression of peripheral retinal NPAs and promote the reperfusion of NPAs. Campochiaro *et al*^[3] observed that monthly injection of 0.3 or 0.5 mg of ranibizumab prevented the worsening of retinal ischemia and promoted the reperfusion of NPAs by blocking VEGF and eliminating the 'positive feedback loop', based on an analysis of prospectively collected data from the BRAVO and CRUISE trials. Monthly injection of ranibizumab also causes slow retinal vessel closure in ME and is associated with retinal reperfusion at the anterior nonperfusion area in

some patients^[4]. Winegarner *et al*^[33] and Levin *et al*^[34] proved that aflibercept may have a positive effect in improving retinal ischemia, maintaining retinal perfusion and even promoting the reperfusion of NPAs; however, the exact mechanism remains unclear. The AFFINITY study revealed that the most significant reduction in nonperfusion was observed in patients with severe NPDR. Reperfusion seems to occur before the development of irreversible retinal capillary damage, and the therapeutic effect of anti-VEGF treatment improved overall retinal perfusion by increasing the stability of the vascular walls^[35].

EFFECT OF ANTI-VEGF TREATMENT ON MACULAR ISCHEMIA IN DR AND RVO

Macular perfusion status is another important indicator for evaluating the effect of anti-VEGF treatment on retinal ischemia. However, many previous large studies of the treatment effects of anti-VEGF on ME did not include the evaluation of MI. Different levels of MI can represent disease severity and progression, and MI is closely associated with VA prognosis. Patients with greater macular perfusion status were more likely to show a better improvement in ME and VA^[19]. Patients with DR usually exhibit enlargement of the FAZ area with varying degrees of MI^[36]. Some studies have found that MI deteriorates after anti-VEGF treatment because blocking VEGF might be harmful to retinal vascular integrity, especially in patients with preexisting retinal ischemia^[24]. Nakamura *et al*^[37] observed that the FAZ areas increased significantly in 33 patients with DME after 3mo of anti-VEGF treatment; in 6% of patients, the FAZ area increased by more than 50%. The deterioration of the macular perfusion index, represented as the area of FAZ increase and the blood flow density decrease, was also found in patients with RVO treated with anti-VEGF therapy^[38-39].

However, an increasing number of studies have shown that MI does not worsen after anti-VEGF therapy in patients with preexisting ischemia. In 2009, Bonini-Filho *et al*^[24] found that there was no change in the extent of MI compared with baseline in all patients at 54wk after anti-VEGF treatment. The Bolt Study and a subanalysis of the RESTORE (Extension) study also revealed that repeated treatment with bevacizumab or ranibizumab did not aggravate macular perfusion deterioration, and anti-VEGF therapy had no harmful effect on capillary integrity^[26,40]. In some studies of quantitative macular perfusion status measured with OCTA, Conti *et al*^[9] also observed that 12mo of intravitreal bevacizumab treatment did not cause macular perfusion index aggravation; Ghasemi Falavarjani *et al*^[41] calculated the changes in FAZ area and vessel density after one anti-VEGF treatment in 18 eyes of 15 patients with DR or RVO and concluded that the FAZ area and vessel density remained statistically unchanged in the short term. These studies demonstrated that anti-VEGF treatment

may be a viable alternative treatment for ME and is safe enough for patients with MI at baseline.

Anti-VEGF treatment might improve MI and promote reperfusion. Gill *et al*^[42] found that the FAZ area decreased significantly after anti-VEGF treatment in patients with DME. Some patients with MI experienced reperfusion of existing macular NPAs after anti-VEGF treatment^[43]. The vascular density in the eyes of patients with RVO was improved 6mo after anti-VEGF therapy accompanied by decreased macular NPAs^[44]. Although many studies have found that anti-VEGF therapy not only has a positive effect on retinal ischemia and MI but also reduces NPAs, these curative effects still need to be confirmed.

REPERFUSION OF NPAS AFTER ANTI-VEGF TREATMENT

It is worth considering whether reperfusion can cause retinal ischemia-reperfusion injury. Nakahara *et al*^[45] observed changes in retinal structure and function induced by retinal ischemia-reperfusion injury; the retinal vascular density was decreased significantly at 7 and 14d after ischemia-reperfusion injury, with pericyte loss after the appearance of endothelial cell degeneration. Rapid neuronal cell damage probably occurred within 2d and released VEGF, leading to capillary degeneration. Since retinal reperfusion occurs slowly, it does not lead to ischemia-reperfusion injury. Retinal reperfusion should be differentiated from collateral vessels because collateral vessels usually form in the acute phase of disease^[46]. Inhibition of VEGF could normalize peripheral cells, stabilize the basement membrane, reopen closed retinal vessels and improve retinal ischemia^[47-48]. In relevant clinical studies, Consigli *et al*^[49] first demonstrated that the calibers of retinal arteries were reduced significantly by aflibercept treatment for DME, which might indicate that anti-VEGF treatment could promote the normalization of abnormal retinal vasculature. Anti-VEGF treatment inhibited the upregulation of VEGF in the retina by decreasing leukocyte aggregation and retinal hyperpermeability^[50].

CONCLUSION

In summary, to date, most studies have determined that anti-VEGF therapy does not aggravate retinal ischemia, but whether anti-VEGF treatment can promote reperfusion in NPAs remains under debate. The development of FA and OCTA technology in recent years has provided a convenient and powerful measurement tool for the evaluation of retinal ischemia.

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