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REVIEW

Progress and Challenges of Anti-VEGF Agents and Their Sustained-Release Strategies for Retinal Angiogenesis

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Abstract: Currently, the treatment for ocular neovascular diseases, including diabetic macular edema (DME) and age-related macular degeneration (AMD), mainly involves repeated intravitreal injection of anti-vascular endothelial growth factor (VEGF) drugs. Although it can preserve vision, repeated injections are an invasive treatment modality, leading to serious complications and reducing patient adherence to treatment. To reduce the frequency of administration, prolong the time of drug action, and avoid repeated intravitreal injections, the combination of sustained-release materials with anti-VEGF drug therapy has become an emphasis in ophthalmology. In this review, we highlight the current state of anti-VEGF technology, its challenges, and the sustained-release strategies under investigation or being used in clinical practice. Both continuous release and considerable therapeutic effects can be achieved by encapsulating anti-VEGF drugs in sustained-release materials to minimize the number of intravitreal injections. At present, two sustained-release materials are being tested in clinical research, and although basic research shows the strong therapeutic application prospects of extended-release drugs, its challenges mainly involve the discrepancy between the release rates in vitro and the efficiency of the drugs in vivo. Briefly, sustained release of anti-VEGF agents is an advantageous strategy for treating retinal angiogenesis.

Keywords: anti-vascular endothelial growth factor, retinal angiogenesis, anti-VEGF drugs, sustained-release strategies, ranibizumab, bevacizumab

Introduction

Currently, the most common blinding fundus diseases worldwide mainly include age-related macular degeneration (AMD),[1] diabetic retinopathy (DR),[2] retinal vein occlusions (RVO),[3] and retinopathy of prematurity (ROP),[4] which cause vision loss at almost all ages and impose a heavy socioeconomic burden. The common hallmark of these diseases is the formation of retinal neovascularization (RNV). The causes of pathological RNV mainly include local or total retinal ischemia, hypoxia, and persistent inflammatory states caused by different influencing factors, which further promote the secretion of proangiogenic cytokines by local cells, such as hypoxia-inducible factor-1α (HIF-1α),[5] vascular endothelial growth factor (VEGF),[5,6] and Platelet-derived growth factor (PDGF).[7] The blood vessels of pathological RNV are more vulnerable to bleeding, causing different degrees of retinal hemorrhage and vitreous hemorrhage, and in severe cases, retinal cells proliferate and form a proliferative membrane, which can eventually lead to blindness.[8] Therefore, the management of these diseases should focus on inhibiting the formation of RNV.

Among the proangiogenic cytokines, HIF-1α as the key mediator of hypoxia is upregulated in the context of aberrant angiogenesis and low oxygen supply in body organs in health and disease.[9] Increased expression of HIF-1α correlates with the promotion of angiogenesis, tumor metastasis, and poor prognosis in multiple types of solid tumors, including

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those of the colon and cervix. Upregulated HIF-1α stimulates the development of new blood vessels, providing nutrients and oxygen to facilitate tumor growth and metabolism, clearing metabolic waste and CO_2.[5] VEGF is a well-established HIF-1 target that plays essential roles in promoting angiogenesis.

VEGF is a highly specific endogenous pro-vascular endothelial cell growth factor. In normal physiologic conditions, VEGF secretion helps to reform endothelial cells in order to construct functional vessels without representing a leaky architecture. VEGF is a crucial target for the inhibition of RNV and the upregulation of VEGF is contributed to the increased vascular permeability, extracellular matrix degeneration, vascular endothelial cell migration and proliferation, and angiogenesis.[10] There are three VEGRs, namely VEGFR-1, VEGFR-2 and VEGFR-3, among them VEGFR-1 and VEGFR-2 are contributed to the formation of new blood endothelial cells, and VEGFR-3 activity is contributed to the formation of new lymphatic endothelial cells.[11] Currently, blocking VEGF activity has become the main strategy for the treatment of neovascular ophthalmopathy. Many surveys have shown that anti-VEGF intravitreal injections can inhibit retinal neovascularization and vascular leakage and improve the visual outcomes in most of these conditions.[12–14]

To reduce the public health burden of fundus neovascular disease, therapeutic approaches including pan retinal photocoagulation, anti-VEGF therapy, Intravitreal injection of steroid and vitrectomy are commonly used clinical strategies.[15–17] Although intravitreal injection of anti-VEGF drugs can inhibit the further formation of retinal neovascularization to a certain extent, it requires one injection per month to achieve an effective concentration of the drug in the eye.[18] Repeated intraocular injections cause a heavy economic and mental burden on patients, and with the increase in the number of injections, the risk of ocular and systemic diseases in patients also increases sharply.[19,20] In addition, some patients showed insensitivity to treatment with anti-VEGF drugs as well as photoreceptor degeneration after long-term repeated treatment.[21] Sustained-release drugs are manufactured to be more potent and slowly dissolved so to release small amounts of drugs into organs or tissues over an extended period.[22] Scientific community is currently exploring controlled release technologies that can prolong the action time of anti-VEGF drugs in the eye. Both continuous release and considerable therapeutic effects can be achieved by encapsulating anti-VEGF drugs in sustained-release materials to minimize the number of intravitreal injections. In this review, we summarize the ocular fundus diseases characterized by pathological RNV, retrospect the development and current state of anti-VEGF agents. Notably, we highlight the sustained release strategies for anti-VEGF drugs under investigation or being used in clinical practice.

Ocular Neovascular Disease

Diabetes Mellitus (DM) and Diabetic Macular Edema (DME)

DM is one of the diseases with a rapidly increasing incidence worldwide and is a serious threat to human health. Currently, 300 million individuals have diabetes worldwide, and the incidence is rising rapidly. Over one-third of diabetic individuals will develop DR, and approximately 10% of these individuals will develop a vision-threatening disease.[23]

DR falls into two broad categories: the earlier stage of non-proliferative diabetic retinopathy (NPDR) and the advanced stage of proliferative diabetic retinopathy (PDR). The classification of NPDR is based on clinical findings manifested by visible features, including microaneurysms, retinal hemorrhages, intraretinal microvascular abnormalities, and venous caliber changes, while PDR is characterized by the hallmark feature of pathological preretinal neovascularization.[23]

An important additional categorization of DR is diabetic DME, which is an important manifestation of DR that occurs across all DR severity levels of both NPDR and PDR and represents the most common cause of vision loss in patients with DR.[24] DME arises from diabetes-induced breakdown of the blood-retinal barrier (BRB), with consequent vascular leakage of fluid and circulating proteins into the neural retina.[23–25] The extravasation of fluid into the neural retina leads to abnormal retinal thickening and cystoid edema of the macula.[26]

Retinal Vein Occlusions

RVO is the second most common retinal vascular disease after diabetic retinopathy.[27] RVO includes central retinal vein occlusion (CRVO), branch retinal vein occlusion (BRVO), and less commonly, hemi-retinal vein occlusion. BRVO is

four to six times more prevalent than CRVO.[28] In BRVO, there is occlusion of a branch of the retinal vein system, while in CRVO, the occlusion is located in the central retinal vein.[27] The estimated prevalence of CRVO worldwide is 2.5 million.[29] RVO is more prevalent in men than women and is more frequent in older age (over 65 years). RVO is associated with increasing age, systemic hypertension, cardiovascular disease, diabetes mellitus, hyperviscosity syndromes, and glaucoma.[28] The most common cause of progressive loss of vision from vein occlusions is macular edema.[30] VEGF is a cytokine released by hypoxic cells. It increases neovascularization and vascular permeability and leads to the development of macular edema in vein occlusions.[31,32]

Age-Related Macular Degeneration

AMD is the leading cause of irreversible vision loss in individuals over 65 years of age. It is estimated that over 187 million people worldwide are affected by the disease.[33] It is characterized by progressive and chronic degeneration of the macula, a central region in the retina measuring 5.5 mm in diameter, responsible for high acuity vision.[34] There are two main types of AMD, namely non-neovascular or dry AMD and neovascular or wet AMD (nAMD). Early AMD is generally asymptomatic and is diagnosed by the detection of drusen deposits of a specific size and number. Approximately 20% of individuals diagnosed with AMD will develop a late-stage disease.[35] Of those who progress to the late stage, 70% will develop neovascular AMD, also known as exudative or wet AMD, and the remainder will progress toward geographic atrophy.[35,36] Neovascular AMD is characterized by abnormal growth of choroidal blood vessels through Bruch's membrane, generally confined below the retinal pigment epithelium (RPE) and/or retina, although it can also penetrate beyond the subretinal space and within the retina, progressing to retinal angiomatous proliferation.[37] These "new" vessels are leaky and proliferative and may eventually lead to fibrosis and scarring, contributing to significant vision loss.[35–38]

Retinopathy of Prematurity

ROP is a developmental retinal vaso-proliferative disease and a leading cause of visual impairment and blindness in children. It is characterized by the growth of abnormal vessels in the incompletely vascularized retina of preterm infants.[39] Globally, in 2010, an estimated 184,700 babies among 14.9 million preterm babies developed at least one stage of ROP, 20,000 of whom became blind (visual acuity < 20/400) or severely visually impaired (visual acuity from < 20/200 to ≥ 20/400) from ROP, and of whom 12,300 others developed mild-moderate visual impairment (visual acuity from < 20/40 to ≥ 20/200).[40] Retinal ischemia causes excessive production of VEGF, which results in neovascularization in severe ROP. Early gestational age at birth, low birth weight, and unregulated oxygen exposure are the main known risk factors for the development of ROP.[41]

Normal retinal vascularization begins at the optic nerve at 16 weeks of gestation and proceeds anteriorly, with full vascularization at 40 weeks of gestation. This orderly developmental process can be disrupted by the abrupt change from an intrauterine to an extrauterine environment in a preterm infant, leading to the development of ROP.[40]

Therapies for Ocular Neovascular Disease

Intraocular treatment modalities for retinal neovascularization include laser photocoagulation, intravitreal injections of anti-VEGF and steroid agents, and vitreoretinal surgery. The laser results in the destruction of cells in the ischemic retina and produce proangiogenic factors such as VEGF, which are responsible for vasoproliferation.[42] A laser is a destructive procedure that damages the peripheral retina and can result in visual field constriction.[43] Although the first-line therapy for most eyes with center-involved DME consists of anti-VEGF injections, intravitreal injections of steroids can also be effective.[24–26] However, intravitreal steroid use is limited by more frequent ocular side effects, such as cataracts and glaucoma.

Since the availability of anti-VEGF agents, there has been a paradigm shift in the management and treatment of retinal neovascularization. Although initially used for the treatment of choroidal neovascularization in neovascular age-related macular degeneration, their application has rapidly spread to other indications as they outperform previously existing treatments. In the past decade, various clinical trials have repeatedly demonstrated superior visual acuity results and outcomes with anti-VEGF treatments.

Features of Anti-VEGF Agents

Anti-VEGF agents, with the effects of reducing RNV and choroidal neovascularization (CNV), and inhibiting vascular permeability, have revolutionized ocular neovascularization therapy. Currently, various anti-VEGF drugs are being used in ophthalmology, mainly including pegaptanib (Bausch & Lomb), bevacizumab (Genentech), ranibizumab (Novartis), aflibercept (Bayer), conbercept (Kanghong), brolucizumab (Novartis), abicipar-pegol (Allergan), faricimab (Roche), KSI-301 (Kodiak Sciences), and ziv-aflibercept (Zaltrap). According to their molecular modalities, these anti-VEGF agents can be divided into aptamers, monoclonal antibodies (mAbs), and recombinant fusion proteins. The timeline of development of anti-VEGF drugs being used in the field of ocular fundus disease is shown in Figure 1.

Pegaptanib

Pegaptanib (Bausch & Lomb, 50kDa) was the first drug to obtain Food and Drug Administration (FDA) approval for intravitreal administration. It is a pegylated aptamer that binds preferentially to the heparin-binding domain of the VEGF165 isoform.[44] Although proven to be efficient in inhibiting the neovascularization process,[45] its molecular features strongly limit its capacity to block VEGF-related pathways, thus making it a subsidiary therapeutic choice.

Bevacizumab

Bevacizumab (Genentech, San Francisco, CA) is a humanized monoclonal antibody of 148 kDa with activity against VEGF, which was first approved by the (FDA) for the treatment of metastatic colorectal cancer.[46] Bevacizumab's mechanisms of action is quite simple; as a pure anti-VEGF antibody, its main effect is to block the neovascular stimulus and VEGF-induced vascular permeability.[47] Furthermore, bevacizumab interacts with HIF-1α, reducing its stimulating effect on VEGF production.[48]

The use of intravitreal bevacizumab dramatically changed the treatment landscape of ocular diseases having VEGF as an etiological factor. Since 2015, bevacizumab has been shown to improve vision in addition to resolving subretinal fluid and decreasing macular edema in patients with neovascular AMD and RVO, respectively.[49] However, there is still no FDA approval of bevacizumab for any intraocular indication given the lack of large randomized clinical trial data. Nevertheless, off-label treatment is within the realm of the accepted "standard of care."

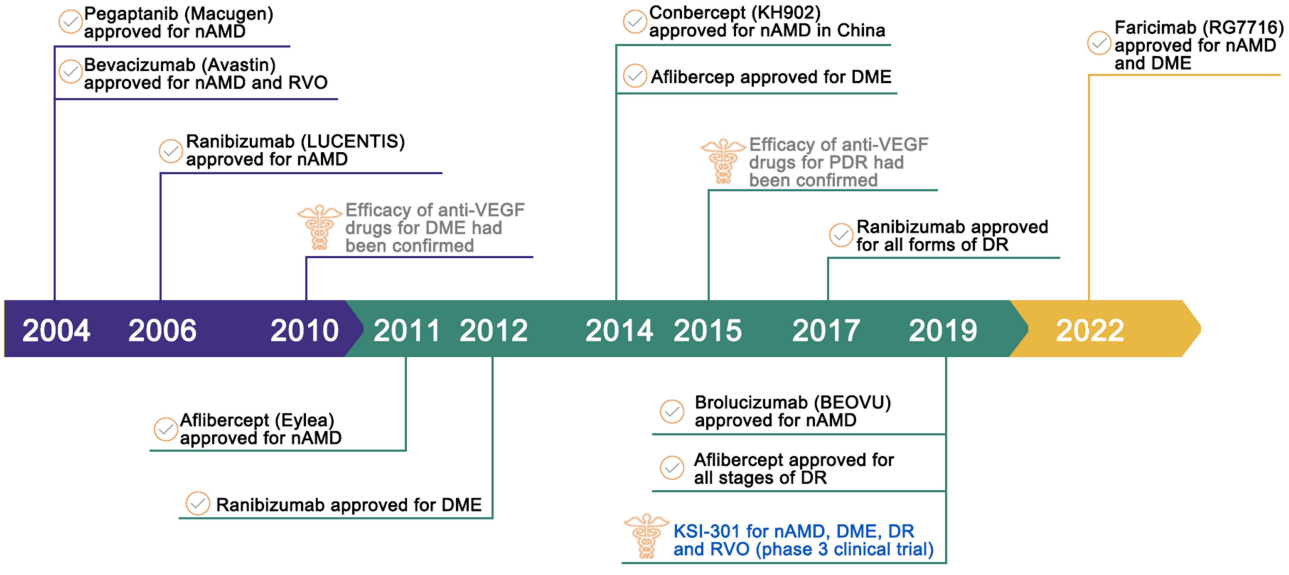

Figure 1 Development timeline of anti-VEGF agents applied in ocular fundus disease. Black: the time indicates the year of FDA approval for anti-VEGF drugs. Blue: the time represents the year of KSI-301 for phase 3 clinical trials. Grey: the time indicates the expanded ophthalmic application of anti-VEGF drugs.
Abbreviations: nAMD, neovascular age-related macular degeneration; RVO, retinal vein occlusion; DME, diabetic macular edema; PDR, proliferative diabetic retinopathy.

Ranibizumab

Ranibizumab (Novartis, Switzerland) is a 48 kDa recombinant humanized monoclonal antibody with affinity toward all isotypes of VEGF-A, inhibiting its biological activity. The lack of a fragment crystallizable (Fc) domain and its small molecule size might expand its affinity for more isoforms of VEGF-A (VEGF165, VEGF121, and VEGF110) and increase its diffusion within the retina and choroid.[50] Furthermore, since ranibizumab has only one binding site for VEGF, two molecules of ranibizumab bind to each VEGF dimer, with the ranibizumab/VEGF-A complex having a higher stability energy than bevacizumab[51] and greater molecular affinity than bevacizumab and aflibercept.[52] It was first approved in the United States for intraocular use in 2006 after two Phase 3 studies (MARINA and ANCHOR) demonstrating its safety and efficacy in limiting vision loss and disease progression in neovascular AMD.[53]

Aflibercept

Aflibercept, also known as VEGF Trap-eye (Eylea®, Regeneron, Rensselaer, NY, USA), is a 115 kDa recombinant humanized protein that acts as a soluble decoy receptor binding to VEGF-A, VEGF-B, and placental growth factor, thereby stopping the binding and activation of VEGF receptors.[54] Since its approval by the FDA for use in nAMD following the landmark VIEW1 and VIEW2 studies,[55] intravitreal aflibercept has been approved for a variety of other pathologies in 2011. There was also a suggestion that injections every 2 months with this VEGF trap may be equivalent to ranibizumab, the gold standard at the time.[56]

Conbercept

Conbercept (Chengdu Kanghong Biotech Company, Sichuan, China) is currently the third most popular molecule belonging to the VEGF Trap family. It consists of a full human DNA sequence of 143 kDa, characterized by the fusion of the extracellular domain 2 of VEGFR1 and the extracellular domains 3 and 4 of VEGFR2 with the Fc portion of human IgG1.[57,58] The pharmacokinetic profile of conbercept is quite similar to that of aflibercept; the main difference is the presence of a portion dedicated to VEGFR2, which was developed to potentially increase the efficacy and stability of conbercept and to produce a relative affinity for VEGF-A, VEGF-B, VEGF-C, and PGF.[57,58]

Brolucizumab

Brolucizumab is a single-chain antibody fragment of 26 kDa, characterized by the absence of the Fc portion, and specifically developed to minimize its molecule size and improve its affinity for VEGF-A isoforms compared with the other molecules.[59] Brolucizumab has been recently approved for use in neovascular age-related macular degeneration, showing noninferiority and higher penetrance within the retina and choroid than the other anti-VEGF molecules.[60]

Abicipar-Pegol

Abicipar-pegol belongs to the family of designed ankyrin repeat protein (DARPins) molecules, a class of molecules that can mimic antibodies and show a high affinity for the VEGF target. More specifically, abicipar-pegol is a recombinant protein of 34 kDa coupled to a polyethylene glycol fraction, binding all VEGF-A isoforms. Its affinity for VEGF-A was found to be comparable to that of aflibercept but remarkably higher than that of bevacizumab and ranibizumab.[61]

Faricimab

Faricimab is the second approved molecule belonging to the DARPin class. The feature of this 150 kDa molecule is that it has two different targets. It can simultaneously and independently bind and neutralize both VEGF-A and Ang-2, the latter enabling interference with the Ang-1/Tie2 pathway.[62] As mentioned above, the Ang-1/Tie2 pathway is a major pathogenic factor in the development of neovascularization and exudation.[63] As a result, faricimab offers an interesting multitarget approach.

KSI-301

KSI-301 (Kodiak Sciences Inc., Nasdaq: KOD, Palo Alto, California) is a new investigational intravitreal anti-VEGF drug of 950 kDa, with a biopolymer of 800 kDa. Its design optimized both its size and molar dose to increase its tissue availability, stability, biocompatibility, potency, and systemic clearance, which in turn are responsible for its durability and design as an optimal ocular PK.[64] KSI-301 blocks all isoforms of VEGF-A and is administered as an intravitreal injection. It has been shown to bind VEGF-A with high affinity (KD 6.75 pM), higher than its cognate receptors VEGFR1 and VEGFR2, and has a high bioavailability in both the retina and choroid/RPE.[65] The ocular tissue half-life has been demonstrated to be > 10.5 days in the retina and > 12.5 days in the choroid in rabbit models.[66]

The clinical results (Phase 1a and Phase 1b) of KSI-301 have thus far shown excellent safety and greater efficacy in three retinal diseases: nAMD, DME, and RVO.[67] The ongoing phase-1b trial has also shown extraordinary biological durability, with most patients achieving treatment-free intervals of 6 months for nAMD, 6 months or longer for DME, and 4 months or longer for RVO.[68] However, these are interim results, and larger trials with long-term follow-up are required to assess the safety, efficacy, and durability of KSI-301 in all three retinal diseases.

Ziv-Aflibercept

Ziv-aflibercept (Zaltrap; Regeneron, Tarrytown, NY, and Bayer Health care, Leverkusen, Germany; 96 kDa) contains the same therapeutic molecule as Eylea but has a much higher osmolarity (1000 mOsm/kg vs 300 mOsm/kg). It was approved in 2012 for the treatment of metastatic colorectal carcinoma.[69] It has recently been used as an intravitreal treatment for various chorioretinal vascular diseases. Several case series have demonstrated that intravitreal ziv-aflibercept is safe and effective as both short-term and long-term therapy.[70] Ziv-aflibercept has been used in several areas of the world because it seems to be as effective as other anti-VEGF drugs and is highly cost effective.[71]

Limitations of Anti-VEGF Therapy

The human eyeball is a spherical structure with a biological barrier that can protect it against foreign substances, but which limits the treatment routes, including through systemic and anterior segment administration. The presence of the BRB, composed of retinal capillary endothelial cells (inner BRB) and retinal pigment epithelial cells (outer BRB), requires a relatively higher initial drug concentration under a nonlocal administration mode.[72] Therefore, the most common administration method of anti-VEGF drugs is intravitreal injection. Depending on the half-life of the drug and the severity of the disease, there are several main clinical treatment protocols: 1) monthly intravitreal injections for 3 months followed by an as-needed reinjection schedule; 2) monthly intravitreal injections for 3 months followed by an as-needed reinjection per month; and 3) monthly intravitreal injections for 3 months followed by an as-needed reinjection every 2 months.[55,73] Some studies have shown that delaying or reducing the number of injections can lead to varying degrees of vision loss.[55,73]

The use of anti-VEGF drugs has changed the outlook for patients with retinal vascular diseases. However, there are concerns regarding the dosage, timing of injection, and long-term functional outcomes of anti-VEGF treatments. Multiple intravitreal injections indicate a long-term duration of treatment and require good patient compliance. Moreover, repeated treatment places a certain economic and psychological burden on the patients, which may further reduce the patient compliance rate. In addition, repeated intravitreal injections can also cause ocular adverse effects, including persistent intraocular pressure (IOP) increases,[74] RPE tears,[75] and geographic atrophy.[76]

Persistent Intraocular Pressure Increase

Serial injections of anti-VEGF agents (ranibizumab or bevacizumab) may lead to a persistent IOP increase.[74] Transient IOP fluctuations may also cause damage to the retinal nerve fiber layer. After a mean of 20 anti-VEGF injections (range, 8–40 injections), the mean IOP was 29.8 mm Hg (range, 22–58 mm Hg), compared with a baseline of 16.9 mm Hg (range, 14–21 mm Hg). Another study showed that in response to the significant acute and transient IOP increase 5 min after each intravitreal injection of anti-VEGF, Bruch's membrane opening (BMO) enlargement, cup widening and

deepening, and prelaminar tissue thinning were observed.[77] Compared with baseline values, significant BMO expansion and retinal nerve fiber layer thinning were observed in the third month. In eyes with more than six intravitreal injections, similar immediate post-injection changes, including IOP increase, prelaminar tissue thinning, and cup deepening, were observed at 1 year, while BMO expansion was not significant.[78]

Retinal Pigment Epithelium Tears

The most common cause of an RPE tear is vascularized retinal pigment epithelial detachment (PED) in patients with exudative age-related macular degeneration. Although RPE tears can develop spontaneously in vascularized PEDs, the most recent cases have been associated with anti-VEGF injections.[75] The subretinal fluid within the PED applies hydrostatic pressure to the RPE and stretches it, and the PED increases as the hydrostatic pressure increases. Contraction of the choroidal neovascular membrane adds tractional forces to the RPE monolayer, especially in larger PEDs.[75]

One observational study retrospectively evaluated the incidence of RPE tears in 72 consecutive patients (74 eyes) treated with intravitreal ranibizumab 0.5 mg for classic CNV. It was found by optical coherence tomography (OCT) and fundus examinations that the incidence of RPE tears in classical CNV patients treated with anti-VEGF was higher than that in untreated patients. The increased risk of an RPE tear after repeated injection of anti-VEGF therapy may be due to increased contraction of the choroidal neovascular membrane.[79] Another study also found that the first RPE tear occurred after an average of 4.5 ± 2.7 injections of anti-VEGF because of neovascular contraction of a type 1 neovascular complex, adherent to the posterior surface of the RPE and spanning a significant portion of the pigment epithelium detachment area. The second RPE tear occurred after an average of 7.1 ± 5.2 injections of anti-VEGF owing to constriction of the fibrous vessels induced by anti-VEGF therapy. Although a "double RPE tear" occurred, as in previous studies, the patient's vision was not significantly affected because the macula was not involved.[80]

Geographic Atrophy

Geographic atrophy secondary to AMD is a progressive and irreversible loss of photoreceptors, RPE cells, and choroidal capillaries. Geographic atrophy develops from intravitreous verruca pigmentation, followed by regression of the intravitreous verruca and pigmentation, and then RPE cell death. Some studies have found that geographic atrophy progresses faster in patients receiving anti-VEGF therapy.[81]

A retrospective review showed that the number of anti-VEGF injections was correlated with geographic atrophy progression, and each additional injection increased the probability of atrophy by 1.35 times. In this study, 72 eyes of 63 patients with exudative AMD were observed, all receiving ischemia-reperfusion injury (IRI) treatment only with a median number of injections of 6.[76] Fundus angiography and imaging examination showed that the cumulative number of anti-VEGF injections was significantly correlated with the progression of atrophy. The logistic regression model showed that each additional injection increased the probability of atrophy by 1.35 times. Similarly, in a treatment trial for exudative AMD, the number of anti-VEGF injections was also confirmed to be correlated with geographic atrophy progression.[82]

Corneal Endothelial Injury and Limbal Mesenchymal Stem Cell (LMSC) Injury

Intravitreal injection is performed on the eye at approximately 3.5 mm to 4 mm behind the limbs, and subconjunctival drug regurgitation can be seen in this area. Anti-VEGF drugs in the aqueous humor are in direct contact with the corneal endothelium and are almost entirely cleared by aqueous circulation from the eyes. Therefore, the risk of corneal endothelial injury associated with repeated intravitreal injections should be considered.

Lass found by specular microscopy that repeated intravitreal aflibercept injection for 52 weeks had no apparent corneal endothelial toxicity in patients treated for neovascular age-related macular degeneration.[83] Currently, there is no definite conclusion about the mechanical effect of intravitreal injection or the long-term effects of anti-VEGF drugs on the cornea. However, additional clinical data is still needed to study the long-term risk because anti-VEGF agents are in direct contact with the corneal endothelium.[84]

Studies have found that LMSCs have the potential to treat corneal injuries, and repeated intravitreal injection of anti-VEGF drugs may reduce LMSC reserves due to their toxic effects. There is a case report of a patient who developed iatrogenic limbal stem cell deficiency due to the intravitreal injection of bevacizumab seven times, indicating that repeated surgical trauma to the limbus and surrounding areas may damage the LMSCs.[85] Moreover, Ugur Acar also confirmed that administration of high doses (5 times and 10 times the clinical dose) or repeated standard doses of intravitreal anti-VEGF agents (aflibercept, ranibizumab, and bevacizumab) may adversely affect the proliferation indices and viability of compact bone derived-mesenchymal stem cells and LMSCs.[86]

Structural Changes in Sclera

Repeated intravitreal anti-VEGF injection may lead to structural changes in the sclera. Zinkernagel assessed the effects of intravitreal anti-VEGF therapy on the scleral structure by spectral domain anterior segment OCT, and patients with surgical trauma from more than 30 intravitreal anti-VEGF injections were found to have a significantly thinner sclera.[87] These results suggest that alternating the injection site should be considered in patients requiring multiple intravitreal injections.

Choroid Vessel Changes

Studies have shown that anti-VEGF therapy reduces the diameter of the retinal and choroid vessels, reduces the thickness of the choroid, and damages the normal choroidal and retinal blood circulation. Anti-VEGF drugs (bevacizumab) exert two successive effects, including vasoconstriction of the retinal and choroidal blood vessels occurs very soon after injection (from minutes to hours) and is related to the link between VEGF and nitric oxide (NO), followed by a decrease in capillary density, which occurs from hours to a few days after injection.[88]

Kim found via swept-source OCT that the thickness of the choroid was significantly reduced in patients treated with multiple intravitreal anti-VEGF injections over 3 months.[89] It was also observed that the choroidal blood flow and velocity decreased in patients with at least two intravitreal anti-VEGF injections (bevacizumab or ranibizumab), but such changes were reversible after drug withdrawal. However, if retinal ischemia is present at the same time, it may lead to serious consequences.[90]

In addition, it was observed that anti-VEGF (aflibercept) treatment with three to five injections can effectively reduce the CNV lesion area but increases the vascular density of the CNV as observed by OCT angiography (OCTA), impacting the blood supply of the choroid.[91] Therefore, for patients with repeated intravitreal injection of anti-VEGF drugs, the vascular morphology and distribution in the fundus should be monitored regularly.

Sustained-Release Devices of Anti-VEGF Agents for Retinal Angiogenesis Treatment

Systemic administration, periocular administration, and intravitreal injection of pharmaceuticals are possible routes of drug delivery to the posterior segment of the eye. Delivery of anti-VEGF agents to the posterior segment presents challenges that arise from the clearance pharmacokinetics and anatomy of the eye. The drug concentration in the eyes is considerably lower than the initial concentration after transfer to the blood circulation. The blood-retinal barrier also leads to low efficiency of the systemic administration route. In addition, the drug may have an adverse impact on other organs.[92]

When using transscleral administration, the drug diffuses through the sclera and circulates to the target tissue of the posterior segment of the eye through the subconjunctival and retrobulbar area, near the posterior sclera or posterior Tenon capsule. Although this method is less invasive, various static barriers of the eye, such as the corneal epithelium, conjunctival epithelium, sclera, choroid, Bruch membrane, and retinal pigment epithelium, and dynamic barriers, including choroidal or conjunctival blood flow, hinder drug diffusion, increase drug loss, and reduce the treatment effects. In addition, the efficiency of drug delivery through the sclera is also affected by the properties of the drug itself, such as charge, hydrophilicity, and molecular weight.[93]

The intravitreal drug delivery method directly delivers the drug to the posterior segment of the eye, effectively avoiding various barrier effects. Therefore, the current guidelines for the treatment of neovascular ophthalmopathy

mostly recommend the use of intravitreal injection of anti-VEGF drugs.[94–97] However, as mentioned earlier, most anti-VEGF drugs have a short half-life, and the effective concentration can only be maintained for approximately 1 month.[98] Even the latest drug to hit the market, broruximab, is extended to only 3 months at an effective concentration in the eye.[60]

To solve the problems of a short duration of action and the low effective concentration of drugs in the eye, researchers have focused on the application of sustained-release devices with anti-VEGF agents to meet the above requirements. The eyeball is an ideal organ for the application of sustained-release devices. The BRB helps to further maintain the intraocular concentration of the drug while minimizing systemic absorption and side effects; at the same time, the eye is also immune-exempt, which limits the occurrence of inflammatory responses associated with sustained-release drug carriers.[99]

Currently, according to the research progress, sustained-release anti-VEGF drugs can be divided into a clinical trial phase and a basic investigation phase. According to the sustained release material, it can be divided into degradable materials and nondegradable materials. According to the delivery route of sustained-release drugs, it can be divided into intravitreal injection administration, scleral implantation, and subconjunctival injection administration. Several sustained-released materials and methods have been studied for anti-VEGF ocular delivery and are summarized in Table 1 and described in the following sections.

Sustained-Release Anti-VEGF Agents in the Clinical Trial Phase

Ranibizumab Sustained-Release Device

The Port Delivery System (PDS) with ranibizumab (Roche/Genentech), the most representative of the non-degradable implants, is currently in phase 3 clinical research for the continuous delivery of ranibizumab.[100–103] The PDS is a permanent, refillable implant inserted through a small incision in the sclera and pars plana. A self-sealing septum in the center of the implant allows the drug to be replenished, thus ensuring that the PDS does not need to be removed from the eye. Ranibizumab moves down the concentration gradient from the implant reservoir by passive diffusion, continuously releasing ranibizumab into the vitreous over time through a porous metal release control element to control the rate of release.[100] The concentration of the drug in the implant gradually decreases over time, and when the concentration of ranibizumab in the PDS implant is 100 mg/mL initially, an effective drug concentration in the vitreous chamber can be achieved, persisting for at least six months.[101]

In a Phase 2 clinical trial, nAMD patients who received 100 mg/mL PDS showed comparable visual and anatomic outcomes but a reduced total number of ranibizumab intravitreal injections compared with those who received monthly intravitreal ranibizumab (0.5 mg) injections both at 9 month and 22 month, suggesting that PDS exhibited the potential to reduce the treatment burden in nAMD while maintaining vision.[100,102] As a phase 3 non-inferiority and equivalence trial demonstrated that the adjusted mean change in best-corrected visual acuity (BCVA) score was +0.2 letters in the PDS arm and +0.5 letters in the monthly ranibizumab arm over 36 weeks. Among the patients enrolled, 98.4% did not receive supplemental treatment in the first 24-week interval. In addition, the PDS implants displayed fewer adverse events within 1 month of implantation.[103] Although the device can reduce the number of intravitreal injections, it still has the problems of poor drug stability and high complications such as endophthalmitis, which need additional larger samples and longer-term safety studies. Moreover, the PDS implants need to be surgically implanted and removed, which reduces the compliance of patients.

GB-102

GB-102 is a tyrosinase inhibitor developed by GrayBug Vision that targets VEGF-A and PDGF. GB-102, the injectable form of sunitinib, forms a depot in the inferior vitreous after being injected, which then gradually releases the drug and biodegrades over time.[104] Studies have shown that a single injection of GB-102 can achieve visual benefits in patients with nAMD after 6 months. In the phase 1/2a clinical study (ADAGIO; NCT03249740), 68% of patients at month 6 had received a single GB-102 injection, and their central subfield thickness (CST) was significantly reduced compared with that before treatment.[104,105] In a subsequent phase 2b clinical study (ALTISSIMO; NCT03953079), CNV lesions secondary to a previously treated nAMD with the same initial dose of GB-102 every 6 months showed comparable

Table 1 Sustained-Release Devices of Anti-VEGF Agents for Retinal Angiogenesis Diseases

Main Sustained-Release Device	Sustained-Release System	Active Molecule	Delivery Route	Degradable	Research Phase	Research Subject	Release Duration	Main Outcomes	Refs.
PDS	PDS	Ranibizumab	Inserted in the sclera and pars plana	No	Phase 2, multicenter, randomized, active treatment-controlled clinical trial	nAMD patients	9 months	1) PDS was well tolerated 2) PDS (100 mg/mL) showed visual and anatomic outcomes comparable with monthly intravitreal ranibizumab injections	[100]
PDS	PDS	Ranibizumab	Inserted in the sclera and pars plana	No	Phase 2, multicenter, randomized, active treatment-controlled clinical trial	nAMD patients	~22 months	1) PDS was well tolerated 2) PDS (100 mg/mL) showed visual and anatomic outcomes comparable with monthly intravitreal ranibizumab injections	[102]
PDS	PDS	Ranibizumab	Inserted in the sclera and pars plana	No	Phase 3, open-label, randomized, visual acuity assessor-masked noninferiority and equivalence trial	nAMD patients	36~40 weeks	1) Over 98% patients did not receive supplemental treatment in the first 24-week of PDS interval 2) PDS showed comparable BCVA outcomes over weeks 36	[101]
GB-102	Bioerodible polymer nanoparticles	Sunitinib	Intravitreal injection	Yes	Phase 1/2, multicenter clinical trial	nAMD patients	6 months	A single dose of GB-102 injection reduced CST of 68% nAMD patients compared with that before treatment	[105]
GB-102	Bioerodible polymer nanoparticles	Sunitinib	Intravitreal injection	Yes	Phase 2b, multicenter, visual examiner-masked, randomized active-controlled clinical trial	nAMD patients	6 months	Every 6 months of GB-102 injection shown comparable BCVA changes and CST changes at month 10 and 12	[106]
PLGA	NPinPMP	Bevacizumab	Intravitreal injection	Yes	Experiment	Rat	4 months in vitro 2 months in vivo	1) Bevacizumab was continuously released from NPinPMP for 4 months in vitro 2) Alexa-bevacizumab from NPinPMP could be detected for 2 months in rat vitreous	[113]

PLGA	Albuminated-PLGA-nanoparticles	Bevacizumab	Intravitreal injection	Yes	Experiment	Rabbit	8 weeks in vitro 56 days in vivo	1) Sustained release of bevacizumab for 8 weeks in vitro 2) Nanoparticles with bevacizumab persistence in rabbit vitreous at day 56	[114]
PLGA	PLGA-PEG-PLGA hydrogel	Bevacizumab	Intravitreal injection	Yes	Experiment	Rat Rabbit	4 weeks in rat vitreous 42 days in rabbit vitreous	PLGA-PEG-PLGA hydrogel sustain released bevacizumab for 4 weeks in rat vitreous and for 42 days in rabbit vitreous	[115,116]
PLGA	PLGA/PCADK blend	Bevacizumab-dextran particles	Intravitreal injection	Yes	Experiment	Rabbit	56 days in vivo	1) PLGA/PCADK sustain released bevacizumab for 56 days in rabbit vitreous 2) PLGA/PCADK-bevacizumab inhibited the vessels proliferation in vivo	[117]
PLGA	PLGA	Ranibizumab biosimilar	Intravitreal injection	Yes	Experiment	HUVEC cells	3 weeks in vitro	Ranibizumab biosimilar encapsulated PLGA continuously release d the drugs for 3 weeks and inhibited HUVEC cell proliferation and tube formation	[121]
Hydrogel	PolyActive™ hydrogel co-polymer	Dual anti-VEGF domain antibodies	Intravitreal injection	Yes	Experiment	Cynomolgus	Over 12 months in vitro 6 months in vivo	1) Sustained release activity in vitro for more than 12 months 2) Anti-angiogenesis in Cynomolgus CNV model for 6 months	[130]
Hydrogel	Hyaluronic acid/dextran-based in situ hydrogel	Bevacizumab	Intravitreal injection	Yes	Experiment	Rabbit	6 months in vivo	Continually released bevacizumab and maintained concentration up to 6 months	[131]
Hydrogel	Hyaluronic acid/dextran-based in situ hydrogel	Anti-VEGF antibody	Intravitreal injection	Yes	Experiment	Rhesus monkeys	At least 5 months	Maintained release in the monkey eye for at least 5 months and preventing the recurrent CNV	[132]
Chitosan	Chitosan-PLGA nanoparticles	Bevacizumab	-	Yes	Experiment	Goat sclera	Not provided	1) Better permeability of bevacizumab across the sclera 2) Longer remaining time on the surface of sclera	[137]

(*Continued*)

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Table 1 (Continued).

Main Sustained-Release Device	Sustained-Release System	Active Molecule	Delivery Route	Degradable	Research Phase	Research Subject	Release Duration	Main Outcomes	Refs.
Chitosan	Chitosan-polyelectrolyte complex	Anti-VEGF antibody	-	Yes	Experiment	HUVEC cells	Over 30 days	1) Sustained release for over 30 days 2) Inhibiting HUVEC cell proliferation and tube formation	[138]
Liposomes	Nanoscale liposomes	Bevacizumab	Intravitreal injection	Yes	Experiment	Rabbit	42 days	Five times higher concentration of bevacizumab released from nanoscale liposomes in rabbit vitreous at day 42	[139]
Liposomes	Bev-MVL	Bevacizumab	Intravitreal injection	Yes	Experiment	Rat	28 days	Maintained therapeutic concentration of bevacizumab in rat vitreous fluids for 28 days	[140]
Light-responsive Nanoparticle	Light-responsive Nanoparticle	Nintedanib	Intravitreal injection	Yes	Experiment	Rat	30 weeks in vitro 10 weeks in vivo	Inhibition of the rat CNV formation within 10 weeks	[144]
BioSilicon	BioSilicon	Bevacizumab	-	No	Experiment	-	20 days	1) The release of bevacizumab can be controlled by adjusting the pore size and surface area of BioSilicon 2) 80% bevacizumab can be released from BioSilicon with pores size of 100nm for 20 days	[145]
PEGDM/COL	PEGDM/COL	Ranibizumab	Episcleral implant	No	Experiment	Rat	18 weeks	Ranizumab can penetrate the choroid and retina from PEGFM/COL through the rat sclera, inhibiting CNV formation for up to 18 weeks	[146]
Polymer solid lyophilized matrix	Polymer solid lyophilized matrix	Bevacizumab	Subconjunctival administration	Yes	Experiment	Rabbit	12 weeks	Comparable effects in the rabbit vitreous up to 12 weeks	[147]

Abbreviations: Refs, references; VEGF, vascular endothelial growth factor; PDS, Port Delivery System; nAMD, neovascular age-related macular degeneration; BCVA, best-corrected visual acuity; CST, central subfield thickness; PLGA, Poly (lactic acid-co-glycolic acid); NPinPMP, nanoparticles in porous microparticles; PLGA-PEG-PLGA, Poly (lactic acid-co-glycolic acid)-poly (ethylene glycol)-poly (lactic acid-co-glycolic acid); PLGA/PCADK, PLGA/poly cyclohexane-1,4-diyl acetone dimethylene ketal; HUVEC, Human umbilical vein endothelial cell; CNV, choroidal neovascularization; Bev-MVL, Bevacizumab-laden polycystic liposomes; PEGDM/COL, photopolymerized poly (ethyleneglycol) dimethacrylate that incorporated collagen microparticles.

BCVA changes and CST changes at months 10 and 12.[106] In addition, animal experiments showed that after a single intravitreal injection, the efficacy of GB-102 in inhibiting VEGF and PDGF could be sustained for up to half a year.[107]

Non-degradable implants can achieve long-term release and show good biocompatibility, but when the drug is depleted, the material needs to be removed by secondary surgery, which increases the risks. Degradable anti-VEGF sustained-release drugs mainly use polymer materials to package anti-VEGF drugs or integrate with the drugs to achieve the purpose of sustained release. After the release of the anti-VEGF drug, the carrier material is gradually degraded in the eye without the need for secondary surgery. Furthermore, the release rate can be adjusted by changing its composition and molecular weight.

Sustained-Release Anti-VEGF Agents in the Experimental Phase

In recent years, due to the disadvantages of non-degradable slow-release drugs, more research focus has been placed on biodegradable slow-release drugs. Anti-VEGF sustained-release drugs mainly use polymer materials to package anti-VEGF drugs or integrate with the drugs to achieve the purpose of sustained release. After the release of the anti-VEGF drugs, the carrier materials will gradually degrade in the eye without the need for secondary surgery. The release rate can be adjusted by changing its composition and molecular weight.

Polylactic-Co-Glycolic Acid (PLGA)

PLGA, one of the most widely studied polymers, is a co-polymer of polylactic acid (PLA) and polyglycolic acid (PGA).[108] PLGA is fully biodegradable and self-assembles into nanomicelles, and as one of the best-defined biomaterials for drug delivery. PLGA can be processed into virtually any shape and size, can encapsulate molecules of virtually any size, and is soluble in a variety of common solvents, including chlorinated solvents, tetrahydrofuran, acetone, and ethyl acetate.[109,110] In water, PLGA is biodegraded by the hydrolysis of ester bonds.[111] Importantly, PLGA has been approved by the FDA for implant applications and is currently widely studied as a biodegradable controlled-release vehicle for drugs.[112] The above physicochemical properties make PLGA an excellent candidate for the intraocular sustained release of anti-VEGF drugs.

In 2013, Sarath K. Yandrapu et al applied supercritical infusion and pressure quenching technology to the preparation of bevacizumab-loaded nanoparticles in porous microparticles (NPinPMP). In vitro, bevacizumab is continuously released from NPinPMP for 4 months, maintaining its monomeric form, conformation, and activity. In addition, the concentration of bevacizumab in the vitreous returned to the baseline level at week 2 after intravitreal injection of bevacizumab in rats. Bevacizumab undergoes sustained release for up to 2 months from bevacizumab-loaded NPinPMP.[113] Albuminated-PLGA-NPs were also used to contain bevacizumab and provided a sustained release of bevacizumab for approximately 2 months. Notably, the bevacizumab concentration in the vitreous of rabbits remained above 500 ng/mL, the minimum effective concentration that completely blocks VEGF-induced angiogenesis for at least 8 weeks.[114] The payload of bevacizumab to PLGA-PEG-PLGA hydrogel or PLAG alone can also fulfill the sustained release of bevacizumab in the rat eye for 4 week[115] and in the rabbit eye for 42 days.[116] Jiaxin Liu et al found that a PLGA/polycyclohexane-1,4-diyl acetone dimethylene ketal (PLGA/PCADK) blend could be loaded with bevacizumab-dextran particles using solid-in-oil-in-water (S/O/W) emulsification, and it exhibited a release behavior of bevacizumab via an increasing total release over 50 days both in vitro and in vivo.[117] Nevertheless, most investigations emphasized the modification and optimization of PLGA materials, aiming to manifest better penetration of the formulation and achieve a higher concentration and an extended release duration in the posterior ocular tissues.

To compare the function of bevacizumab-encapsulated PLGA and bevacizumab, XP Zhang et al used an alkaline burn-induced corneal neovascularization model and an oxygen-induced retinopathy model to demonstrate that bevacizumab-loaded PLGA improved the anti-angiogenesis capability for handling both corneal and retinal neovascularization.[118] In recent years, considerable evidence has highlighted the critical role of immune inflammatory processes in the pathogenesis of retinal and choroidal neovascularization.[119] Accordingly, dexamethasone, a widely used corticosteroid for treating diabetic retinopathy, ocular inflammation, and CNV, was loaded into PLGA/PEI nanoparticles (DPPNs), and bevacizumab was subsequently adsorbed onto the DPPNs (eBev-DPPNs) by static electricity. eBev-

DPPNs presented excellent antiangiogenic efficiency compared with dexamethasone or bevacizumab both in vitro and in a rabbit CNV model.[120]

PLGA is currently the most broadly studied anti-VEGF agent sustained-release material, of which bevacizumab-loaded PLGA is the most widely investigated in retinal and choroidal neovascular diseases. There are also studies that combined ranibizumab biosimilar with PLGA particles. Release experiments in vitro showed that ranibizumab biosimilar could be continuously released from PLGA particles for several weeks, and the ability of ranibizumab-encapsulated PLGA to inhibit vessel proliferation was comparable to that of ranibizumab.[121]

Hydrogel-Based Drug Delivery Systems

Hydrogels are an ideal class of material for sustained-release drugs in addition to polymerizing implants and colloidal drug delivery systems.[122–125] Hydrogels are composed of a three-dimensional network of hydrophilic polymer chains with high water retention capacity, the properties of which can be changed by monomers and crosslinkers.[126] Due to these properties of hydrogels, they have been successfully applied to soft contact lenses (SCLs), intraocular lenses, and artificial tear fluids, and the use of hydrogels as sustained-release carriers to deliver anti-VEGF drugs has also increased rapidly.[127] Similar to the challenges of PLGA designs, drug sustained-release hydrogels occupy only a small portion of the vitreous space and typically degrade over time and release the drug, without necessarily requiring excellent optical transparency. As a sustained-release drug system, hydrogels can effectively reduce the number of intraocular injections, thus helping to reduce the risk of endophthalmitis, retinal detachment, and lens injury caused by invasive procedures. In addition, the porous structure of the hydrogel guarantees that it can store higher doses of the drug than the free-drug form.[127,128]

To be able to control and promote sustained drug release, researchers have developed a variety of sophisticated methods to optimize the formulation of hydrogels. In 2017, Xu et al used four poly (amidoamine) dendrimers to cross-link with PEG and dibenzocyclooctyne groups to promote azide-alkyne cycloaddition to form a hydrogel by strain.[129] In a study using primates, the researchers applied microparticles of PolyActive^{TM} hydrogel copolymer as an anti-VEGF delivery base, and two different anti-VEGF domain antibodies (dAb) attached to a human IgG1 Fc region served as dimeric anti-VEGF molecules. The efficacy results showed that this system had a lower IC50 than aflibercept and maintained sustained release activity in vitro for more than 12 months. In vivo studies have shown that this delivery system can exert a protective effect in a cynomolgus CNV model for up to 6 months after a single intravitreal injection.[130]

During the same period, Yu et al implemented catalyst-free chemical crosslinking between vinylsulfone-functionalized hyaluronic acid (HA-vs) and thiolated dextran (Dex-SH) to form an in-situ hydrogel. Mixed bevacizumab, HA-vs, and Dex-SH were injected into rabbit vitreous, and it continually released bevacizumab and maintained its concentration for up to 6 months, 107-fold higher than a bolus injection.[131] Three years later, Yu's team used the same formula of this anti-VEGF controlled-release hydrogel system to prove that a relatively constant concentration of anti-VEGF drug can be maintained in the monkey eye for at least 5 months and that the dosage was sufficient to prevent recurrent CNV after a single injection.[132]

Overall, hydrogel sustained-release vehicles can achieve longer anti-VEGF drug release than PLGA delivery systems, and their function has been demonstrated in CNV models of primates, although evidence from clinical studies is still lacking.

Chitosan

Chitosan is a natural cationic polymer with the characteristics of hydrophilia, biocompatibility, biodegradability, and mucoadhesivity.[133–135] Chitosan has similar structural characteristics to specific parts of the extracellular matrix, enabling easier carry of drugs. In addition, chitosan presents a variety of shapes, including nanoparticles, thin films, and hydrogels. Chitosan covalently cross-linked hydrogels have a stable mesh structure and are an ideal biomedical drug carrier material.[136] Subconjunctival injections and subconjunctival delivery patterns are becoming potential methods for posterior ocular tissue therapy. Based on nanotechnology, chitosan-coated PLGA nanoparticles (NPs) of bevacizumab were prepared. Compared with the drug solution, chitosan-coated NPs demonstrated better permeability of bevacizumab across the sclera and a longer remaining time on the surface of the sclera, thereby improving the drug residence time and permeability. Therefore, bevacizumab-loaded chitosan-coated NPs can be used as candidate carriers for the subconjunctival injection of anti-VEGF drugs.[137] The chitosan polyelectrolyte complex (PEC) system, containing polysaccharides

alginate and chitosan, can produce a 30-day delivery of anti-VEGF antibodies and inhibit VEGF-induced endothelial cell proliferation and angiogenesis in vitro.[138]

Liposomes

Liposomes are small vesicles made of lipid bilayers that can carry hydrophilic and lipophilic drugs simultaneously. Bevacizumab was encapsulated in nanoscale liposomes, and the concentration of free bevacizumab in the eyes receiving liposome bevacizumab was five times higher at 42 days compared to rabbit eyes with glass cavity injection of soluble bevacizumab.[139] There are also been studies of bevacizumab-laden polycystic liposomes (Bev-MVL) that more effectively maintain the therapeutic level of bevacizumab in vitreous fluids for a longer duration than bevacizumab solutions (Bev-S) for 28 days in rat CNV.[140]

Light-Responsive Nanoparticle

Conventional anti-VEGF sustained-release drugs are released according to the sustained-release characteristics of the drug design after injection into the vitreous cavity, and their release cannot be controlled after injection. One class of drugs is prepared using triggered carriers that can be administered with high spatial and temporal resolution through external triggering. Taking advantage of the photoreceptor system of the eyeball, it is theoretically feasible to use light to control the drug release, and an injectable implant (ISFI) formed in situ in response to the light is a photosensitive liquid that can be injected into the vitreous body in a minimally invasive manner. Through noninvasive light irradiation of the cornea, it quickly changes its physical properties to form a transparent gel to achieve sustained release and improve the bioavailability of the drug.[141]

Studies have demonstrated the possibility of using light to control nanoparticles targeting cells and tumors. In studies using photosensitive nanoparticles to treat CNV, intravenous administration and intravitreal administration have both been reported,[142,143] such as vitreous cavity photosensitive anti-VEGF nanoparticles, which stably retain encapsulated angiogenesis inhibitor (nintedanib) molecules in the vitreous and can trigger drug release for up to 30 weeks after injection by exposure to far ultraviolet light (UV) on demand, and animal experiments have also confirmed that it can inhibit the formation of rat CNV for up to 10 weeks.[144] The advantage is that light-responsive nanoparticles can trigger the on-demand release of the drug in a noninvasive manner in vitro, but there are also safety issues such as potential damage from the trigger source (far-ultraviolet radiation) to other tissues of the eye.

Others

BioSilicon, an oxidized porous silicon, was prepared with bevacizumab as its substrate. The release of bevacizumab can be controlled by adjusting the pore size and surface area of BioSilicon. Bevacizumab (80%) can be released from BioSilicon with a pore size of 100 nm for 20 days.[145]

Photopolymerized poly (ethylene glycol) dimethacrylate incorporating collagen microparticles (PEGDM/COL) was developed as an episcleral implantable device for the sustained release of ranibizumab. By implanting the device through the sclera, ranibizumab can penetrate the choroid and retina through the rat sclera, inhibiting CNV formation for up to 18 weeks.[146]

In addition, Burgalassi et al produced a polymer solid lyophilized matrix containing bevacizumab. A comparative study in rabbit eyes showed that the injection of lyophilized matrix under the conjunctiva could be used as a posterior ocular delivery system of bevacizumab, which is less invasive and has a longer drug release time.[147]

Conclusion

In this review, we have summarized several retinal vascular diseases characterized by RNV as the main pathological feature and listed the current research status and the challenges of anti-VEGF drugs with FDA approval or those still in the research phases, as well as studies on the most common sustained-release materials of anti-VEGF drugs to address these challenges and dilemmas.

Although researchers are continuously trying to develop a variety of anti-neovascular therapies that are more efficient, have a longer half-life, and have safer and noninvasive delivery methods, the development and research of sustained-

release strategies for existing anti-VEGF drugs seem to be able to address the limitations of current anti-VEGF drug treatments faster and better. The advantages of sustained-release dosage forms of anti-VEGF agents include: 1) long-term therapeutic effects by continuous release of the medicine; 2) reduces frequency of dosing; 3) prolonged action of the medicaments; 4) control of drug therapy; 5) ability to modify the extent and rate of drug absorption; 5) improves patient compliance.

According to the recent publications, the most well-studied sustained-release materials include degradable and non-degradable materials. Degradable materials are now widely preferred by researchers because there is no need for secondary surgery to remove them. However, it's worth noticing that it is necessary to receive other intraocular injections of anti-VEGF agents if the degradable materials were degraded before completely controlling disease progression. The main advantage of non-degradable materials is that they can be repeatedly supplemented with drugs. The operation of supplementary drugs is simpler compared with intraocular injection, and as long as the implant material does not cause side effects on the eye, it can be placed there for a long time. Thereby ensuring maximum prolongation of the action time of anti-VEGF drugs. In addition, light-responsive nanoparticle also shows unique advantages for ocular applications, with the potential of triggering the on-demand release of the drug in a noninvasive manner. What can not be ignored is light-responsive strategies may cause the potential damage from far-ultraviolet radiation to tissues of the eye.

In terms of the anti-VEGF drugs carried in different forms, bevacizumab is the most commonly used drug, followed by ranibizumab, probably because these two drugs may have been used for the treatment of RNV diseases for a relatively longer time. With the ideal therapeutic effects of ranibizumab in ophthalmic research field, the combination of ranibizumab and sustained-released materials may provide convenience and benefits for patients.

The main concern for the application of sustained-released strategies is the effective duration. Most of the strategies can reach a release time of at least one and a half months, PDS shows a continuous release of anti-VEGF drugs for 1 year, and hydrogel-carried anti-VEGF drugs can also achieve sustainable release in the vitreous cavity of primates for six months to one year and can achieve therapeutic effects. Regarding to the research progress, the investigations on PDS and GB-102 are significantly ahead of other drugs and have entered the phase 2 and 3 clinical research phases.

Notably, most of the investigations are in the basic research stages. The use of PLGA has been reported more frequently, and hydrogel research has used primates as its main model to explore the effects of sustained-release anti-VEGF drugs. We should also note that some studies of sustained-release materials have focused more on demonstrating long-term drug release in vitro rather than evaluating the effects of anti-VEGF itself. Whether the mechanisms of sustained-released anti-VEGF drugs were changed needs more proofs.

The main problem facing the research is how to select and prepare the drug carrier, which can meet the requirements of stable combination with anti-VEGF drugs, long released periods, enhanced intraocular penetration and good biocompatibility. Furthermore, whether in basic or clinical studies, the abundance of anti-retinal angiogenesis drugs has shown surprising and promising outcomes. Studies on the administration of anti-VEGF with sustained-release materials are showing increasingly good progress. In the future, with advances in the development of nanotechnology, new materials, and the cooperation and complementarity among different research fields, we will achieve more efficient and safer therapeutic strategies for ocular fundus diseases.

Abbreviation

DME, diabetic macular edema; AMD, age-related macular degeneration; VEGF, vascular endothelial growth factor; RNV, retinal neovascularization; PDS, port delivery system; PLGA, polylactic-co-glycolic acid; DR, diabetic retinopathy; RVO, retinal vein obstruction; ROP, retinopathy of prematurity; HIF-1α, hypoxia-inducible factor-1α; PDGF, platelet-derived growth factor; DM, diabetes mellitus; NPDR, nonproliferative diabetic retinopathy; PDR, proliferative diabetic retinopathy; DME, diabetic macular edema; BRB, blood-retinal barrier; CRVO, central retinal vein occlusion; BRVO, branch retinal vein occlusion; RPE, retinal pigment epithelium; CNV, choroidal neovascularization; FDA, Food and Drug Administration; nAMD, neovascular age-related macular degeneration; IOP, intraocular pressure; BMO, Bruch's membrane opening; PED, pigment epithelial detachment; IRI, ischaemia-reperfusion injury; LMSC, limbal mesenchymal stem cell; OCT, optical coherence tomography; OCTA, OCT angiography; BCVA, best-corrected visual

acuity; CST, central subfield thickness; PLA, polylactic acid; PGA, polyglycolic acid; NPinPMP, nanoparticles in porous microparticles; PLGA/PCADK, PLGA/polycyclohexane-1,4-diyl acetone dimethylene ketal; S/O/W, solid-in-oil-in-water; DPPNs, PLGA/PEI nanoparticles; SCLs, soft contact lenses; NPs, nanoparticles; ISFI, injectable implant; UV, ultraviolet light.

Data Sharing Statement

All data generated or analyzed during this study are included in this published article and are available from the corresponding author, Prof.Xiaorong Li (email address: xiaorli@163.com), on reasonable request.

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Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare that they have no competing interests.

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