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EDITORIAL

Pharmacologic vitreolysis: New strategy for treatment of anomalous vitreo-macular adhesion

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Abstract

Persistent anomalous vitreo-macular adhesion (VMA) is a well-known factor, associated with a variety of sight threatening diseases - including macular hole, vitreo-macular traction syndrome, cystoid and diabetic macular edema, exudative age- related macular degeneration, myopic traction maculopathy and others. With the advent of optical coherence tomography our understanding of these pathologies and the ability of their early diagnosis has gone much far in the past two decades. The release of macular traction has been of exclusive surgical capability. Notwithstanding good results, vitrectomy is hampered by the inability of complete vitreo-retinal separation (*i.e.*, smooth, bare internal limiting membrane), compulsory postoperative positioning in macular hole cases, surgical complications, and high costs. With aim to offer less invasive and safe treatment modality for anomalous VMA, investigators have made enormous progress in the past decade. Leading among the studied nonsurgical measures is the intravitreal application of pharmacologic agents for the induction of vitreo-retinal separation and vitreous liquefaction, a method termed pharmacologic vitreolysis. Several vitreolytic agents have been studied to date, the most potent among them proved to be plasmin. Recently, ocriplasmin (formerly known as microplasmin) - a more stable than plasmin recombinant product, proved to be safe and efficient in releasing VMA in large studies, and consequently received FDA approval. It's role in clinical practice is now in the process of being determined. This paper aims to review and summarize the current knowledge and status of investigation on this new approach for the treatment of VMA.

Key words: Pharmacologic vitreolysis; Vitreo-macular adhesion; Posterior vitreous detachment; Macular hole; Microplasmin

Core tip: Persistent anomalous vitreo-macular adhesion (VMA) is a well-known factor, associated with a variety of sight threatening diseases (macular hole, vitreo-macular traction syndrome, macular edema, exudative age-related macular degeneration). The release of

traction has been of exclusive surgical capability. Notwithstanding good results, vitrectomy is hampered by the inability of complete vitreo-retinal separation and surgical complications. With aim to overcome limitations of surgery, investigators have made enormous progress with the advent of pharmacologic vitreolysis - a method for releasing VMA by intravitreal drug delivery. This paper aims to summarize the current knowledge and status of investigation on this new treatment approach.

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INTRODUCTION

With the advent of optical coherence tomography (OCT) - a sophisticated modality for retinal imaging, ophthalmologists obtained more knowledge on the important role of the posterior vitreous in a variety of retinal diseases. In the development of physiologic, or age-related, posterior vitreous detachment (PVD) two processes (liquefaction - synchysis, and fibrillar collapse - syneresis) take place simultaneously and interact, thus resulting in vitreo-retinal separation[1-3]. With time areas of liquefaction increase, the collagen meshwork fibrils form thick fibers (synergetic debris), and after separation from the internal limiting membrane (ILM) the posterior hyloid collapses anteriorly[1-3]. While previously we believed the process of PVD to be an acute one, recent OCT studies have shown that it is a gradual one and may take ears. Usually PVD starts as a shallow separation of the hyaloid from the retina in the perifoveal area and expands gradually until the last detachment from the optic disc margin. The results of this last separation are acute symptoms and the sign of Weiss ring (complete PVD)[4,5]. In some subset of eyes this physiologic process of complete PVD is hampered by firm vitreo-retinal adhesions to different sites - optic disc margin, fovea, or focal areas in retinal periphery. If this is the case, the dynamic traction of the posterior hyaloid exerted upon retina at points of adhesion gives rise to various complications, such as vitreous hemorrhages, macular hole, vitreo-macular traction syndrome (VMT), vitreo-papillary traction syndrome, retinal tears and retinal detachment. It has been documented that persistent vitreo-macular adhesion (VMA) may aggravate macular edema and retinal pathology in various conditions such as diabetic retinopathy (DR), retinal vein occlusions, neovascular age-related macular degeneration (AMD), uveitis, myopic maculopathy, and others[6-8]. Persistent vitreo-retinal adhesions may serve as scaffold for vitreo-retinal neovascular proliferations in DR and retinal vein occlusions. Sebag and associates have revealed the role of vitreoschisis (vitreous cleavage with residual vitreous cortical layer on retinal surface) for the pathogenesis of macular holes and epiretinal membranes (ERM)[3].

The therapeutic option in all these pathologic vitreo-retinal entities for many years has been vitreo-retinal surgery. Notwithstanding good results[9,10], vitrectomy is hampered by the inability of complete vitreo-retinal separation (*i.e.*, "smooth", "cell-free" ILM, ILM)[11], compulsory postoperative positioning for macular hole cases, surgical complications, and high costs. Some studies draw our attention that after vitrectomy, despite meticulous PVD induction and thorough aspiration, or posterior hyloid peeling, some cortical vitreous fibers may still remain and adhere to the retinal surface, and thus give rise to fibrocellular proliferation and formation of postoperative ERM[12]. Gandorfer and coauthors have documented by electron microscopy and immunocystochemistry that in 2/3 of vitrectomy cases with ERM removal, cortical vitreous cells remain on the ILM, which subsequently lead to recurrence of ERM[11]. To achieve a "cleaner" retinal surface, surgeons may peel the ILM in every case, but this increases the risks of some complications, such as nerve fiber layer damage, retinal haemorrhages or breaks, and paracentral scotomas. With aim to overcome limitations of vitrectomy, investigators have explored as alternative different methods for achieving complete PVD and "smooth" ILM. Leading among the studied nonsurgical techniques is the application of different pharmacologic agents in the vitreous for inducing vitreo-retinal separation and vitreous liquefaction. This method was termed pharmacologic vitreolysis by Sebag[13]. As a result of a huge work in this field of ophthalmology by many investigators, such as Sebag, Gandorfer, de Smet, Stalmans and others, we have now a better understanding of vitreo-macular pathology and recently obtained pharmacologic vitreolysis in the treatment armamentarium for anomalous VMA in our clinical practice. The early interest of vitreolysis was concentrated on the use of vitreolytic agents in difficult cases for obtaining cleaner vitreo-retinal separation (pharmacology assisted vitrectomy)[13,14]. Realizing the potential of vitreolysis, investigators have then begun to explore the use of vitreolytic substances as stand-alone drug deliver therapy for the treatment of anomalous VMA related diseases[15,16]. This paper aims to review and summarize the current knowledge and status of investigation on this new treatment approach.

VITREOLYTIC AGENTS

Pharmacologic vitreolytic substancies can be categorized according to the mechanism of action as "enzymatic" (plasmin, microplasmin, tissue plasminogen activator, nattokinase, chondroitinase, dispase, and hyaluronidase) and "non-enzymatic"(Vitreosolve and RGD peptides - arginine-glycine-aspartate peptides). Sebag[17,18] offers a more useful classification, based on their biological effect - "liquefactants" (able to induce liquefaction),

"interfactatns" (able to disrupt vitreo-retinal adhesions) or having both effects. Sole liquefactants are collagenase and hyaluronidase, sole interfactants are RDG peptides and dispase, and having both effects - chondroitinase, nattokinase, plasmin, microplasmin, tissue plasminogen activator, and Vitreosolve.

It must be stressed, that for the induction of safe PVD with complete vitreo-retinal separation, it's fundamental to achieve both effects. If liquefaction occurs without adequate vitreo-retinal interface disruption, this will result in worsening of the existent tractional pathology[17,18].

Collagenase

Collagenase is a bacterial protease, purified from Clostridium histolyticum and it selectively cleaves collagen type Ⅱ which comprises the fibrillar meshwork of the vitreous body[19]. It acts as a sole liquefactant. In animal models collagenase succeeded to liquefy the vitreous, but was noted to have adverse effects - ILM damage, disruption of retinal architecture, and retinal toxicity proved by histological and electrophysiological examination[20]. In recent studies of collagenase-assisted pars plana vitrectomy some complications have been noted - vascular digestion of proliferative membranes and retinal hemorrhages[21].

Hyaluronidase

Hyaluronidase represents an endoglycosidase which is able to dissolve hyaluronan - a molecule that comprises the glycosaminoglycan meshwork of the vitreous body. Hyaluronidase is a pure liquefactant and its' effect was demonstrated *in vitro*[22] and *in vivo*[23], and recently in a phase Ⅲ trial (Vitrase) in the management of hemophthalmus[24]. As it has no effect on vitreo-retinal adhesions, if applied alone may worsen existing VMA-related pathologies.

Dispase

Dispase represents a protease molecule which cleaves collagen Ⅳ and fibronectin, and thus attenuates attachments between the hyaloid and the ILM. In experimental *in vivo* animal studies some harmful effects were reported - retinal toxicity with disruption of ganglion cells and photoreceptor layers, retinal and vitreous hemorrhages, cataract and lens subluxation[23].

RGD peptides

Integrins are receptor molecules on the cell surface which take part in the cellular - extracellular matrix signaling and adhesion. They are bound to the ILM by a specific sequence of amino acids - RGD (arginine-glycine-aspartate). Synthetic RDG peptides compete for integrin-biding sites and thus disrupt the integrin-extracellular matrix interaction and loose vitreo-retinal adhesions[25]. RGD peptides are non-enzymatic and are considered as pure interfactants. In a rabbit model RGD peptides facilitated the induction of PVD during vitrectomy, and no toxicity was noted[26]. No further investigations are reported.

Vitreosolve®

Vitreosolve® (Vitreoretinal Technologies Inc, United States) is a non-enzymatic urea-based molecule that is considered to have both liquefatant and interfactant vitreolytic effects. It currently undergoes Phase Ⅱ/Ⅲ study in patients with non-proliferative DR without PVD. Preliminary results demonstrate good ability at achieving complete PVD. Final results are being expected.

Chondroitinase

Chondroitinase is a protease which catalyzes depolymerization of chondroitin sulfate, hyaluronan, and dermatan sulfate. It has both liquefactant and interfactant properties. The results from pre-clinical studies are mixed. One group found no significant effect on inducing PVD[20], while another group reported complete vitreo-retinal disinsertion in a monkey model[27]. High doses demonstrate some toxicity, while lower doses were unable to achieve significant rates of spontaneous PVD, or bare ILM after viteo-retinal separation[28].

Nattokinase

Nattokinase is a serine protease produced by Bacillus subtilis and is derived from fermented soybean. It is known to have fibrinolytic effect and is under investigation in cardiovascular and thrombotic therapy. It is considered to enhance the activation of plasmin by increasing the synthesis of tissue plasminogen activator (tPA), thus it has both liquefactant and interfactant properties[29]. In a rabbit model nattokianse showed good vitreolytic property with leaving smooth ILM, but only in the highest intravitreal doses tested. These doses, however showed also adverse actions, such as alterations in retinal structure, intraretinal hemorrhages, and toxicity confirmed by electroretinography[30].

Plasmin

Plasmin represents a serine protease which lyses laminin, fibrin, and fibronectin, and also acts through increasing the levels of other proteases that disrupt extracellular matrix structures. Its' primary action is to weaken vitreo-retinal adhesion, and to a less extent provoke liquefaction[31,32]. Plasmin was the most widely studied vitreolytic agent, and in many pre-clinical studies has shown good properties in achieving complete PVD with bare ILM (in a dose-dependent manner), and its' safety profile was excellent[33-37].

However, plasmin is extremely unstable. The application of plasmin in clinical practice requires activation of plasminogen (its' proenzyme) with plasminogen activators immediately prior to use. As there is no commercially available plasminogen, investigators rely on a very expensive and time-consuming process of generation of autologous human plasminogen derived

from patients' own plasma and purified *via* affinity chromatography[37]. Numerous studies using the described technique in difficult vitrectomy cases with plasmin-assisted PVD, such as retinopathy of prematurity (stage 5)[38], tractional DME, complicated proliferative DR[39], complicated X-linked retinoschisis[40] report ease in PVD induction, improved final anatomic outcomes, and no enzyme-related complications[37-40]. However, this method is quite expensive, time-consuming and inapplicable in daily clinical setting.

Plasminogen activators (tPA and urokinase)

Plasminogen activators have fibrinolytic properties and are approved for non-ophthalmic vascular disorders (stroke, symptomatic coronary artery). They exert their effect through plasmin, thus having potent viteolytic properties. Their advantages are commercial availability, safety in terms of microbal contamination (recombinant molecule), established ocular safety in some other ophthalmological conditions (post-surgical fibrin lysis, submacular hemorrhage, acute retinal vein occlusion)[41,42]. Pre-clinical studies on plasminogen activators for inducing PVD show promising efficacy and safety results[43,44]. The difficulty in applying plasminogen activators in clinical practice comes from the inability to achieve sufficient quantities of intraocular plasminogen (which can be achieved by blood-retinal barrier brake down, *i.e.,* cryopexy), or exogenous administration. Thus dosing would be imprecise.

Ocriplasmin (microplasmin)

Ocriplasmin (formerly known as microplasmin) represents a recombinant protein which contains the catalytic domain of plasmin, and so having the properties of human plasmin[45]. Microplasmin was developed for intravenous administration for the treatment of systemic thromboembolic disease. Its' effects after intravitreal application are specific for vitreous and less active on ocular structures, such as vessels, lens, lamina cribrosa, and ciliary body[46]. It has numerous advantages over plasmin, autologus plasminogen, and tPA: it is more stable than plasmin, commercially available, allows accurate dosing, generated by recombinant technique it assures sterility, the smaller size (22 kDa of microplasmin versus 88 kDa of plasmin) facilitates its' permeability in tissues. Pre-clinical studies have demonstrated a dose- and time-dependant efficacy in achieving complete PVD with clean, bare ILM[32,33,46]. It showed no histological or functional toxicity, except a- and b-wave depression in electroretinography in cases, treated with the highest dose (250 μg)[47].

The most potent and safe vitreolytic agent among all tested proved to be microplasmin, thus it underwent exploration in a series of clinical trials sponsored by ThromboGenics and collectively entitled the Microplasmin Intravitreal Injections (MIVI) trials - 14 listed in the clinical trials registry. The majority has been completed and ocriplasmin (Jetrea, ThromboGenics Inc) received FDA approval (on 17th October 2012) for nonsurgical treatment of symptomatic VMA.

MIVI Ⅰ was an uncontrolled Phase Ⅰ/Ⅱa clinical trial that aimed to assess the safety profile and efficacy of ocriplasmin, applied intravitreally in different concentrations (25, 50, 75, and 125 μg) and increasing exposure times (2 h, 24 h and 7 d). Subjects of the trial were patients scheduled for surgery (with DME, VMT syndrome, macular hole)[48]. The incidence of spontaneous PVD as well as the ease of PVD induction during vitrectomy was found to be dependent on the dose and time exposure. However, less than 50% of eyes in every subgroup developed spontaneous PVD. Except one case of retinal detachment, there was no safety concern described[48]. The results from this initial trial have demonstrated the good safety profile of ocriplasmin and confirmed that it's capable in inducing PVD in some cases.

MIVI Ⅱt (traction) was a prospective and sham-controlled Phase Ⅱ clinical trial for assessment of the efficacy of ocriplasmin alone for the treatment of symptomatic VMA and macular holes. Four cohorts were examined in randomization 4:1 to ocriplasmin at doses 75, 125, 175 μg and sham[49]. The primary endpoint of non-surgical release of VMA at day 28 after injection was reached in 8%, 25%, 44% and 27% of patients in the sham, 75, 125, 175 μg cohort, respectively. The greatest proportion of VMA release was noted until day 7, and repeated injections in eyes with unreleased VMA after day 28 in the 125 μg cohort did not increase the chance of PVD induction.

MIVI Ⅲ was a larger multicenter prospective placebo-controlled study designed to evaluate three doses of ocriplasmin (25, 75, and 125 μg) compared to placebo for facilitating PVD before vitrectomy[50]. The percentage of complete PVD were 10%, 14%, 21% and 31% for the placebo, 25, 75, and 125 μg ocriplasmin, respectively.

MIVI-TRUST comprises pooled data from two parallel multicenter, randomized Phase Ⅱ clinical trials (MV 006 and MV007), which had same protocol except the ratio of randomization. The aim was to compare a single dose of 125 μg ocriplasmin with sham in patients with symptomatic VMA alone and in VMA associated with macular hole[51]. The primary endpoint of VMA resolution at day 28 was achieved in 26.5% of ocriplasmin treated eyes and in 10% of placebo-injected eyes ($P < 0.001$). Non-surgical closure of macular holes resulted in 40.6% of ocriplasmin treated eyes compared to 10.6% of sham-injected eyes ($P < 0.001$). The subgroup analysis showed that resolution of VMA at day 28 was achieved more often in eyes without ERM, younger patients (< 65 years), eyes with full thickness macular hole, phakic eyes, and those with a focal VMA ≤ 1500 μm[52]. Eyes with macular hole width ≤ 250 μm were more likely to achieve nonsurgical macular hole closure. As safety concerns, investigators reported: similar rates of retinal holes (0.9% *vs* 1.6%) and retinal detachment (1.1% *vs* 2.7%) in the ocriplasmin and vehicle

injected eyes, respectively; decrease in visual acuity with > 3 lines in 5.6% and 3.2% in the ocriplasmin and sham injected eyes (a condition of progression of the pathology, that requires proper monitoring and timely schedule for surgical treatment); mild transient intraocular inflammation in 7.1% and 3.7% of eyes injected with ocriplasmin and sham, respectively; 2% of ocriplasmin cases reported dyschromatopsia and accompanying a-and b-wave amplitude decrease in electroretinography; potential for lens subluxation[51,52].

Studies for treatment of anomalous VMA in cases with DME (MIVI 11), ARMD (MIVI 5), as vitreolysis-assisted vitrectomy in children and infants scheduled for surgery (MIC), and in uveitic macular edema (MIME) are still undergoing and their results are being expected.

The use of ocriplasmin is now on its' way of translation to the real world clinical practice. Ophthalmologists report comparable results to those in the clinical trials[53,54], or even better in cohort of selected (best outcome expectancy) cases[55]. Singh and coauthors report overall response rate of 47.1% (8/17 eyes), in patients meeting three of four positive predictors criteria (*e.g.,* focal VMA ⩽ 1500 μm, no ERM, and phakic lens status) they report successful VMA release in 50.0% (7/14 eyes), and patients meeting all four criteria (*e.g.,* VMA diameter ⩽ 1500 μm, no ERM, younger than 65, and phakic lens status) showed a response of 75.0% (3/4 eyes)[55]. Other authors have published initial results of much lower macular hole closure rate - 12.5% (one of 8 eyes with stage 2 macular hole)[56], unsuccessful resolution of VMA (none of 7 treated eyes)[57], and enlargement of macular hole with worsening of visual acuity[58]. With view of previous good results and the latter disappointing ones, a careful selection of candidates for ocriplasmin treatment as well as watchful observation after treatment should be done. It is important to discuss with the patient that in rare cases macular hole progression may result with worsening of the condition. On the whole, investigators that are involved in the development of ocriplasmin treatment, advise that candidates for ocriplasmin injections should be scheduled for surgery, thus if drug delivery does not succeed within 4 wk, surgery would be performed without delay.

In terms of adverse effects ophthalmologists report their clinical observations of vision loss[59,60], dyschromatopsia, subretinal fluid accumulation predominantly in cases with release of VMA[61], cystoid macular edema development[62], spectral OCT detection of disturbances in the neuroreceptor ellipsoid zone[60-64], as well as documented by electroretinography a decrease in the a-and b-waves[63,64]. These effects seem to be short (months)[59] or long lasting (years)[60], but transient. These documented observations raise the concern about the enzymatic effect on photoreceptors and pigment epithelial cells. Further investigations are needed to elucidate the precise mechanisms by which ocriplasmin exerts these retinal microstructure alterations.

CONCLUSION

Though great progress has been done in the research process, the development of non-surgical treatment for anomalous VMA related diseases is very much an ongoing work. From the various agents, tested for the needs of pharmacologic vitreolysis, microplasmin has shown the greatest potential for safe and complete PVD. Randomized controlled clinical trials documented efficacy, but in less than 50% of cases. In selected cases (smaller than 250 μm macular holes, without ERM, focal VMA ⩽ 1500 μm, younger than 65, and phakic lens status) the prognosis is documented to be better, thus they represent best candidates for ocriplasmin treatment. Safety results seem satisfactory, though caution regarding some possible complications is advisable. The clinical role of ocriplasmin in cases with macular traction and persistent DME, uveitic edema, exudative AMD and others is still under investigation.

Future perspectives in this field of research would cover exploration of non-enzymatic agents that would offer vitreolysis without collateral damage of adjacent structures. Some investigators believe that the most promising concept would be to use a mixture of specific agents at much lower doses, previously found to have some toxicity, as a combination therapy may allow the use of lower and safer doses to increase the success rate of VMA release.

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EDITORIAL

Approved pharmacotherapy for macular edema secondary to branch retinal vein occlusion: A review of randomized controlled trials in dexamethasone implants, ranibizumab, and aflibercept

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Abstract

There are three approved pharmacotherapies for treating macular edema secondary to branch retinal vein occlusion (BRVO), including corticosteroids (dexamethasone implants) and anti-vascular endothelial growth factor (VEGF) (ranibizumab and aflibercept). They all show superior ability to improve vision and reduce macular thickness, comparing with sham injections or macular grid laser treatment. There is no severe ocular or systemic adverse reaction reported in studies associated with anti-VEGF for macular edema after BRVO. Intraocular pressure elevation and cataract aggravation should be addressed after intravitreal dexamethasone implants. Single intravitreal dexamethasone implant had effective duration as long as four to six months. Intravitreal anti-VEGF requires six monthly injections as loading doses, and then PRN regimen needed according to functional and anatomical changes. Ozurdex and ranibizumab reduce not only macular edema, but also the probability of retinal ischemia and neovascularization in patient s with BRVO. Prompt treatment with these agents can lead to a better outcome.

Key words: Branch retinal vein occlusion; Intravitreal injection; Aflibercept; Ranibizumab; Macular edema; Ozurdex

Core tip: There are three approved pharmacotherapies

for treating macular edema secondary to branch retinal vein occlusion (BRVO), including corticosteroids (dexamethasone implants) and anti-vascular endothelial growth factor (VEGF) (ranibizumab and aflibercept). They all show superior ability to improve vision and reduce macular thickness, comparing with sham injections or macular grid laser treatment. There is no severe ocular or systemic adverse reaction reported in studies associated with anti-VEGF for macular edema after BRVO. Intraocular pressure elevation and cataract aggravation should be addressed after intravitreal dexamethasone implants. Single intravitreal dexamethasone implant had longer effective duration than two anti-VEGFs.

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Branch retinal vein occlusion (BRVO) is a common sight-threatening retinal vascular disorder, in which macular edema is the main cause of visual impairment[1]. The pathophysiology of macular edema involves both the presence of inflammation and angiogenic stimulant regarding vascular endothelial growth factor (VEGF)[2,3]. Intravitreal injections of anti-VEGF, including ranibizumab[4-7], bevacizumab[8], pegaptanib[9], aflibercept[10] are proven to be effective for treating macular edema resulting from BRVO. Intravitreal injections of corticosteroids, potent anti-inflammatory agents, such as dexamethasone implants[11-13] and triamcinolone acetonide[14], have been shown to be beneficial to macular edema associated with BRVO. The Food and Drug Administration of United States and European Medicines Agency have approved intravitreal injections of dexamethasone implants, ranibizumab, and aflibercept for treating macular edema secondary to BRVO. Herein the clinical outcome of the randomized controlled studies in these approved pharmacotherapies will be reviewed.

Ozurdex™ (Pharm Allergan Inc., Irvine California) was the first intraocular implant that could slowly release dexamethasone. Ozurdex showed an anti-edematous effect as early as 7 d after implantation[14]. The effect can persist as long as four to six months after single injection[11,12]. The GENEVA study, a randomized controlled trial, collected 291 eyes with BRVO receiving Ozurdex 0.7 mg, 260 eyes in Ozurdex 0.35 mg, and 279 eyes in sham injections[11]. Following single intravitreal injection of Ozurdex 0.7 or 0.35 mg, maximal response was found two months after the injection with visual improvement in nearly ten letters, significantly better than five-letter gain in the sham group. The central retinal thickness also showed significant decrease in the treatment group than in the sham group 90 d after Ozurdex implantation. The effect of Ozurdex diminished six months after the injection. The same response for macular edema was noted after repeated injections of Ozurdex during 12-mo follow-up[12]. Over 12 mo, cataract progression occurred in nearly one third of phakic eyes, and a 10-mmHg intraocular pressure increase from baseline was observed in 15.4% of all patients receiving two injections of Ozurdex 0.7 mg. The intraocular pressure increases were usually transient and controlled with medication or observation. A laser or surgical procedure to reduce intraocular pressure was required for only 14 study eyes. IOP required specific time for clinical monitoring[15]. The dexamethasone implants were reported migration into the anterior chamber, causing permanent corneal edema[16]. Absence of lens capsule and prior vitrectomy were risk factors for Ozurdex anterior migration[16]. In eyes with BRVO in the GENEVA study, longer macular edema duration at the time of first Ozurdex treatment was associated with a significantly lower likelihood of achieving clinically meaningful improvements in vision or macular thickness 6 or 12 mo after treatment[17]. This suggests that prompt Ozurdex treatment may be associated with improved clinical outcomes[17]. The proportion of BRVO eyes with active neovascularization increased from baseline to day 180 in the sham group, but stayed relatively constant in the Ozurdex-treated group in the GENEVA study[18]. It is hypothesized that corticosteroids are associated with the down-regulation of the VEGF and inhibition of ocular neovascularization.

The SHASTA study was a multicenter retrospective study collected 157 patients with macular edema secondary to BRVO[19]. The patients received intravitreal Ozurdex 0.7 mg injection as monotherapy or with adjunctive treatments. Mean reinjection interval was 5.6 mo. Two third of the patients achieved more than 2-line visual improvement in the peak response. Intraocular pressure increase more than 10 mmHg occurred in one third of patients, but only 1.7% of patients required incisional glaucoma surgery. Another randomized multicenter study compared clinical outcome of Ozurdex monotherapy and Ozurdex combined with macular grid laser in patients with macular edema associated with BRVO[20]. The combination of Ozurdex implant and macular grid laser was synergistic for visual improvement and lengthening the time between Ozurdex injections.

Ranibizumab (Lucentis™, Genentech, Inc., South San Francisco, CA, and Novartis Pharma AG, Basel, Switzerland) is an antibody fragment with a high binding affinity towards all forms of VEGF-A, which can effectively inhibit intraocular level of VEGF-A. The BRAVO study included 397 patients with macular edema after BRVO, who were randomized 1:1:1 to receive 6 monthly intraocular injections of 0.3 or 0.5 mg of ranibizumab or sham injections[4]. At month 6, ranibizumab 0.3 or 0.5 mg resulted in a mean gain of 16.6 and 18.3 letters, significantly better than 7.3 letters in the sham group. The central foveal thickness also demonstrated significant decrease in the treatment group than in the

sham group. No significant ocular or nonocular safety events were identified. All the patients including the sham group received PRN ranibizumab injections from month 6 to month 12[5]. The mean number of intravitreal ranibizumab was nearly three injections in the treatment group between month 6 and month 12. At month 12, ranibizumab 0.3 mg or 0.5 mg resulted in a mean gain of 16.4 and 18.3 letters, significantly better than 12.1 letters in the sham group. In the HORIZON trial, 304 patients with BRVO treated with PRN ranibizumab administration according to the protocol of the BRAVO study completed 2-year follow up[6]. The mean number of intravitreal ranibizumab was 2.1 injections in the 0.5 mg ranibizumab group between month 12 and month 24[6]. At month 24, ranibizumab 0.5 mg injection caused a mean gain of 17.5 letters, which maintained the visual outcome comparing to the results at month 6 and month 12. Fewer ranibizumab injections were required to control the edematous condition from month 6 to month 24. In the RETAIN study, 34 BRVO eyes treated with ranibizumab according to the protocol of the BRAVO study completed 4-year follow up[7]. Half of the patients required frequent injections, and another half of them had edema resolution without further treatment. There was a trend that the patients with resolved macular edema had more visual improvement in 25.9 letters, compared with those with unresolved edema in visual gain of 17.1 letters. The retrospective analysis of the BRAVO study suggest that initiating ranibizumab injection immediately after diagnosis of BRVO provides greater vision gain than the patients receiving delayed treatments[21]. Another analysis of the patients with BRVO in the BRAVO study found 79.1% (0.3 mg) and 84.7% (0.5 mg) having central foveal thickness less than 250 μm 3 mo after treatment, and therefore was categorized as early ranibizumab responders[22]. The early ranibizumab responder demonstrated better visual outcome at months 6 and 12, comparing to late or incomplete responder[22]. After analysis of the data in the BRAVO trial, ranibizumab injections prevent the worsening of retinal nonperfusion area, and even promotes reperfusion of the ischemic area, comparing to the sham group[23].

Aflibercept (Eylea™, Regeneron Pharmaceuticals, Inc., and Bayer Pharma AG, Berlin, Germany) is a decoy receptor fusion protein, composed of the second domain of human VEGF receptor 1 and the third domain of VEGF receptor 2, which are fused to the Fc domain of human IgG1. Aflibercept can downregulate both VEGF-A and placental growth factor, which are synergistic for pathologic angiogenesis. The VIBRANT study, a randomized controlled trial, demonstrated the efficacy of intravitreal aflibercept 2 mg over the macular grid laser for 183 patients with macular edema associated with BRVO[10]. The authors used monthly injections for 6 mo[10]. The 6-mo results showed the aflibercept group gained mean 17.0 letters, significantly better than the laser group having only mean 6.9-letter improvement. Decrease of macular thickness was more prominent in the aflibercept group than in the laser group, without accompanying serious ocular and systemic adverse events.

Although there was no serious adverse effect reported in studies of ranibizumab and aflibercept for macular edema secondary to BRVO, some rare serious complications were found after use for other indications. Retinal pigment epithelium tears, macular ischemia, cataract progression, retinal breaks and detachment, endophthalmitis, macular hole, and intraocular inflammation were reported as ocular complications after intravitreal anti-VEGF for treating neovascular AMD[24]. Systemic adverse effects were uncommonly reported such as thromboembolic events (stroke and myocardial infarction) and gastro-intestinal bleeding[24].

In summary, there are three approved pharmacotherapy for treating macular edema secondary to BRVO, including intravitreal injections of corticosteroids (dexamethasone implants) and anti-VEGF (ranibizumab and aflibercept). They all show superior ability to improve vision and reduce macular thickness, comparing with sham injections or macular grid laser treatment. There is no severe ocular or systemic adverse reaction reported in studies associated with anti-VEGF for macular edema after BRVO. Intraocular pressure elevation and cataract aggravation should be addressed after intravitreal dexamethasone implants. Single intravitreal Ozurdex had effective duration as long as four to six months. Intravitreal anti-VEGF requires six monthly injections as loading doses, and then PRN regimen needed according to functional and anatomical changes. Ozurdex and ranibizumab reduce not only macular edema, but also the probability of retinal ischemia and neovascularization in patients with BRVO. Prompt treatment with these agents can lead to a better outcome.

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REVIEW

Ocular renin-angiotensin system with special reference in the anterior part of the eye

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Abstract

The renin-angiotensin system (RAS) regulates blood pressure (BP) homeostasis, systemic fluid volume and electrolyte balance. The RAS cascade includes over twenty peptidases, close to twenty angiotensin peptides and at least six receptors. Out of these, angiotensin Ⅱ, angiotensin converting enzyme 1 and angiotensin Ⅱ type 1 receptor (Ang Ⅱ-ACE1-AT1R) together with angiotensin (1-7), angiotensin converting enzyme 2 and Mas receptor (Ang(1-7)-ACE2-MasR) are regarded as the main components of RAS. In addition to circulating RAS, local RA-system exists in various organs. Local RA-systems are regarded as tissue-specific regulatory systems accounting for local effects and long term changes in different organs. Many of the central components such as the two main axes of RAS: Ang Ⅱ-ACE1-AT1R and Ang(1-7)-ACE2-MasR, have been identified in the human eye. Furthermore, it has been shown that systemic antihypertensive RAS- inhibiting medications lower intraocular pressure (IOP). These findings suggest the crucial role of RAS not only in the regulation of BP but also in the regulation of IOP, and RAS potentially plays a role in the development of glaucoma and antiglaucomatous drugs.

Key words: Angiotensin converting enzyme 1; Angiotensin converting enzyme 2; Angiotensin converting enzyme-inhibitors; Angiotensin Ⅱ; Angiotensin (1-9); Angiotensin (1-7); Glaucoma; Intraocular pressure; Renin-angiotensin system

Core tip: Many of the central components of renin-

angiotensin system (RAS) have been identified in different structures of the human eye. Recent findings suggest that local RAS accounts for long term changes in ocular tissue level. Antihypertensive drugs which inhibit RAS (Angiotensin converting enzyme or AT-receptor blockade) reduce intraocular pressure suggesting their possibility as anti-glaucomatous drugs in the future. Here we describe the local intraocular RAS especially in the anterior part of eye.

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INTRODUCTION

Glaucoma is after cataract the second leading cause of vision loss worldwide. In 2020, 79.6 million people are estimated to be diagnosed with glaucoma. The majority of these patients are estimated to have open angle glaucoma[1]. Glaucoma is a neurodegenerative disorder that leads to the loss of the axons of the optic nerve and to the death of retinal ganglion cells by non-apoptotic and apoptotic mechanisms all of which in the end cause visual field defects and irreversible vision loss[2-6]. Together with age and family history, increased intraocular pressure (IOP) is one of the known major risk factors for glaucoma[2,6,7]. In subjects with increased IOP, ocular hypotensive medication prevents or delays surgery of glaucoma[8]. A 30% reduction in IOP reduces disease progress 10%-35% in glaucoma patients[9,10]. Even though risk factors and possible outcomes of glaucoma are known, the exact mechanism behind development of glaucoma is still poorly known. Interestingly, imbalances in the local ocular renin-angiotensin system (RAS) cascade have been associated to glaucoma[3].

In addition to the circulating RAS that controls blood pressure (BP) homeostasis, electrolyte balance and systemic fluid volume, tissue-specific RAS, accounting for local effects and long-term changes in tissue level, have been described. Local RA-systems have been demonstrated in different organs studied[11,12], including the human eye[2,12-14]. Systemic antihypertensive drugs which inhibit RAS can reduce IOP. Certain ACE inhibitors[15] and AT1 receptor blockers[16] have been shown to reduce IOP in both non-glaucomatous and glaucomatous patients. In animal studies angiotensin converting enzyme (ACE) inhibitors[17,18], AT1 receptor blockers[19,20], and renin inhibitors[21] have been reported to lower IOP. These findings imply that RAS is not only important in the regulation of BP but that it is possibly also involved in the regulation of IOP[5,22]. However, the question of how RAS is involved in the regulation of IOP remains to be answered.

In this review we describe the tissue RAS cascade and concentrate on the anterior part of the eye. A survey of PubMed using the following keywords was performed to collect the literature on eye, IOP (38214, number of reports), RAS (26697), tissue RAS (4870), angiotensin (110705), angiotensin I (7879), angiotensin Ⅱ (55855), angiotensin converting enzyme (45777), angiotensin (1-9) (28), angiotensin (1-7) (1043), Mas receptor (305), angiotensin receptor (16021), eye disease (4830), glaucoma (55288), diabetic retinopathy (DR) (25958), retinopathy of prematurity (ROP) (5710) and age-related macular degeneration (10875). Combining the used keywords allowed to narrow down the literature to 185 references which were used in this review. They were selected based on the abstracts.

RAS: CIRCULATING RAS AND TISSUE RAS

History

The very first clue of the existence of RAS was found in 1898 when scientists Robert Tigerstedt and Per Bergman in Finland discovered that injecting renal homogenate from one rabbit to another causes an acute elevation of BP indicating that kidney secretes a vasopressor substance, named renin[23,24]. Due to the discovery of this hormone, RAS was first thought to be a hormone system through which the kidney influences systemic cardiovascular regulation[25]. Over 40 years later more RAS effectors were found. In 1940, groups working under Braun-Menéndez and Page reported that previously identified renin catalyzes the formation of pressor peptide, first named angiotonin or hypertensin, from a plasma protein substrate angiotensinogen[22,26,27]. Later angiotonin was renamed angiotensin[22].

In the early 1970s major components of the circulating RAS were found and its important role as a BP and fluid balance regulator was understood[23]. In addition, first antihypertensive medications were developed in the 1970s. First of these drugs was captopril, an ACE inhibitor that was designed to prevent the formation of vasoconstrictive peptide Angiotensin (Ang) Ⅱ[22,23]. In 1988, Ang Ⅱ receptor type 1 blockers (ARBs) were invented which main goal was to prevent the direct effects of Ang Ⅱ mediated through angiotensin Ⅱ type 1 receptor (AT1R)[12]. During past years many new peptides and a new angiotensin reseptor type (Mas receptor, MasR) have been identified. MasR is an important member of the RAS, and its actions are mainly opposite to those of AT1R. Mas-receptors play a role in cell proliferation and antifibrosis as well as vasodilatation and local fluid volume homeostasis. In fact, the potentials of MasR ligands, like Ang (1-7) and ACE2 in degrading vasoconstrictive Ang Ⅱ to vasodilatory peptides are regarded as a present focus of cardiovascular drug development[28-30].

Circulating RAS

When RAS was first described, it was seen as a linear cas-

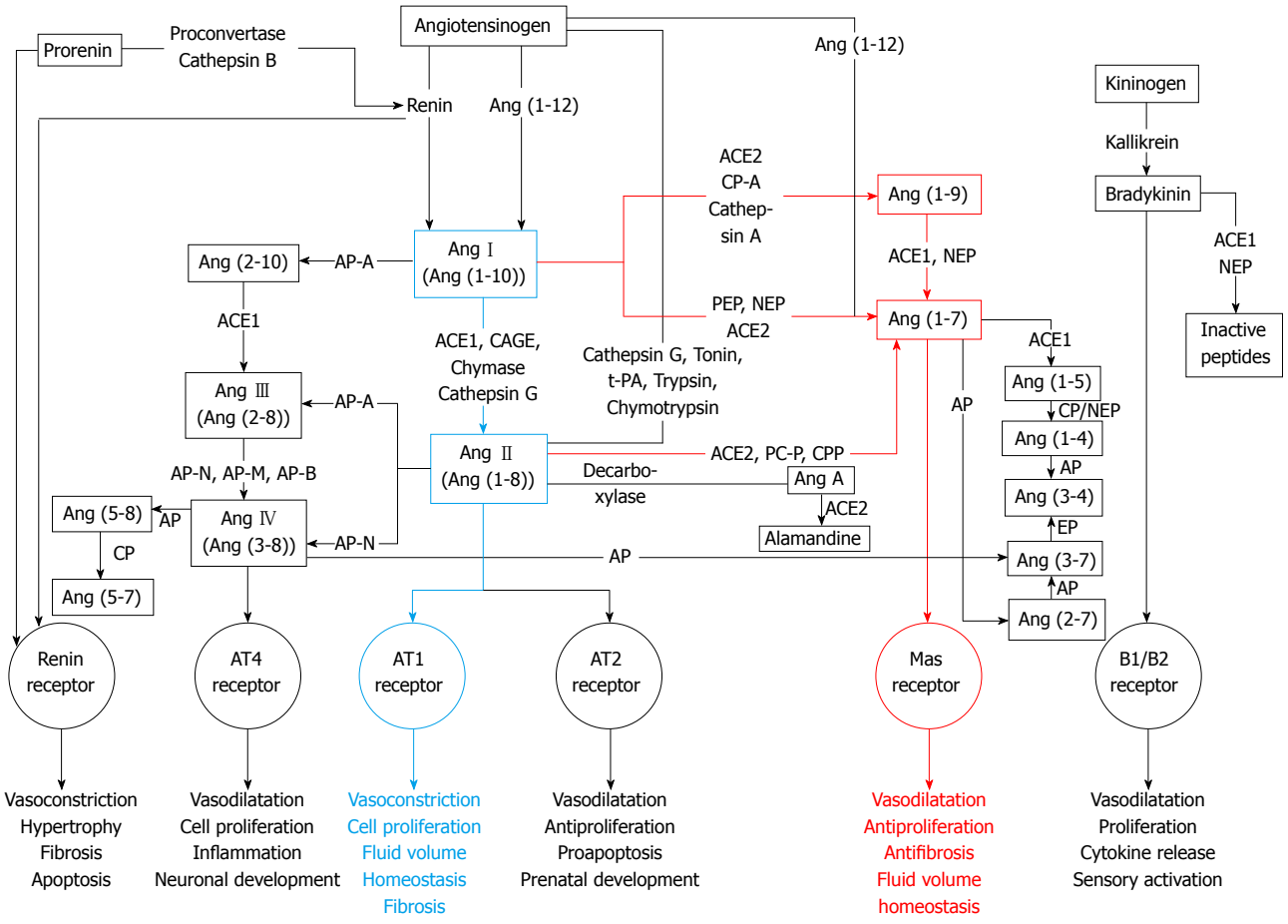

Figure 1 The renin-angiotensin system. The two main pathways of RAS: Ang II-ACE1-AT1R (blue lines) and Ang(1-7)-ACE2-MasR (red lines) are highlighted with colours. ACE(1): Angiotensin-converting enzyme (1); ACE2: Angiotensin-converting enzyme related carboxypeptidase; Ang I, II, III, IV: Angiotensin I, II, III, IV; Ang(1-10): Angiotensin (1-10); Ang(1-8): Angiotensin (1-8); Ang(2-8): Angiotensin (2-8); Ang(3-8): Angiotensin (3-8); Ang(1-9): Angiotensin (1-9); Ang(1-7): Angiotensin (1-7); Ang(1-5): Angiotensin (1-5); Ang(1-4): Angiotensin (1-4); Ang(2-7): Angiotensin (2-7); Ang(3-7): Angiotensin (3-7); Ang(3-4): Angiotensin (3-4); Ang(1-12): Angiotensin (1-12); Ang(5-8): Angiotensin (5-8); Ang(5-7): Angiotensin (5-7); Ang(2-10): Angiotensin (2-10); Ang A: Angiotensin A; AT1R: Angiotensin II type 1 receptor; AT2R: Angiotensin II type 2 receptor; AT4R: Angiotensin II type 4 receptor; AP: Aminopeptidase (-A, -N, -M, -B); B1/B2: Bradykinin receptors; CAGE: Chymostatin-sensitive AngII generating enzyme; CP: Carboxypeptidase; EP: Endopeptidase; Mas receptor: Ang(1-7) receptor type; Nep: Neprilysin; PEP: Prolyl endopeptidase; PCP: Prolylcarboxypeptidase; tPA: Tissue-type plasminogen activator. The picture is updated from Vaajanen *et al*[160].

cade consisting of only one substrate (angiotensinogen), two proteases (renin and ACE1), two peptides (AngI and Ang Ⅱ) and one receptor (AT1R). Today, RAS is known to consist of several enzyme pathways and to include over twenty peptidases, close to twenty angiotensin peptides and at least six receptors[31,32]. Thus, the classical linear cascade has evolved to a cascade with multiple mediators, multifunctional enzymes and multiple different receptors mediating the effects of angiotensin peptides[33-35]. The complexity of the RAS cascade known today is seen in Figure 1.

Central peptides of RAS

Angiotensinogen (AGT) is a 255 amino acids long α-glycoprotein that is synthesized in and released from liver. Renin catalyzes the reaction in which angiotensinogen is converted into AngI[22,36,37]. Mainly synthetized in the liver, angiotensinogen is also formed in heart, vessels, kidney and adipose tissue[38]. The synthesis of α-glycoprotein angiotensinogen is stimulated *e.g.* by inflammation, insulin and estrogens[36].

Angiotensin Ⅰ (Ang Ⅰ), a weak active prohormone, also known as angiotensin (1-10), is a decapeptide generated from angiotensinogen by an enzyme renin[39]. Ang Ⅰ, a weak vasoconstrictor is further cleaved to an octapeptide Ang Ⅱ by ACE1 removing two amino acid residues (His-Leu) from the carboxy terminal of AngI[39,40]. Ang Ⅱ can also be generated by enzymes other than ACE1 such as chymase and cathepsin G.

Angiotensin Ⅱ (Ang Ⅱ), also known as Ang(1-8), first isolated in 1940 and characterized as a potent vasoconstrictor that elevates BP[26,27]. Then, RAS was regarded as an endocrine system in which circulating Ang Ⅱ regulates electrolyte balance, vascular tone, thirst, water intake, aldosterone synthesis, sympathetic activity, sodium handling in the kidney, and antidiuretic vasopressin release from the posterior part of hypophysis[37]. In circulating RAS, renin formed in the kidney is the rate-limiting factor for Ang Ⅱ formation whereas in vascular tissue ACE1 and chymase are the main actors in Ang Ⅱ generation[41].

Ang Ⅱ exerts its main actions *via* two types of

receptors, AT1R and AT2R[36,42]. Ang Ⅱ can be generated from Ang Ⅰ by three different categories of enzymes: ACE1, a metallo dipeptidyl carboxypeptidase, secondly aprotinin-sensitive serine proteases, such as trypsin, tonin, kallikrein and cathepsin G and thirdly a group of chymostatin-sensitive serine proteases, such as human chymase[43]. Ang Ⅱ, a potent vasoconstrictor stimulates the release of vasopressin and aldosterone and thus participates sodium and water retention all of which act in concert to raise BP[37]. ACE inhibitors as antihypertensive medication block the conversion of Ang Ⅰ to Ang Ⅱ by ACE1, thus antagonizing the harmful effects of Ang Ⅱ on AT1R[36].

Angiotensin Ⅲ (AngⅢ), also known as Ang(2-8), is generated from Ang Ⅱ or from angiotensin (2-10) by aminopeptidase A and ACE1[22,23,36,37]. This heptapeptide was found in 1970s and it exerts its actions *via* AT1 and AT2 receptors. AngⅢ has higher affinity to AT2 receptors than to AT1 receptors[44]. AngⅢ induced vasoconstriction and release of aldosterone are close to those of Ang Ⅱ. AngⅢ has 40% of the vasoconstriction activity of Ang Ⅱ[22,23,37]. In some actions on AT1R the role of AngⅢ is at least equally important as that of Ang Ⅱ[23,37].

Angiotensin Ⅳ (AngⅣ), is generated from Ang Ⅱ by aminopeptidase N or from AngⅢ by several other aminopeptidases N, M and B[22,37]. This hexapeptide [Ang(3-8)] exerts its actions *via* angiotensin Ⅱ type 4 receptor (AT4R) found in kidney, lung, brain and heart[23,45,46]. However, AngⅣ can also induce its effects such as renal vasodilatation, hypertrophy and regulation of cell growth in endothelial cells, cardiac fibroblasts and vascular smooth muscle cells by interacting with AT1R[47]. Furthermore, AngⅣ is thought to have an important regulatory role in cardiovascular damage, cognition and renal metabolism and it might be involved in the vascular inflammatory response[22,37].

Angiotensin (1-9) [Ang(1-9)] is formed by cleaving one amino acid residue from the carboxyl terminus of AngI by ACE2[48] and is metabolized by ACE1 and NEP to generate Ang(1-7)[49]. Ang(1-9) can also be generated from Ang Ⅰ through the activity of carboxypeptidase A or cathepsin A[50,51]. The formation of Ang(1-9) is dependent on ACE2 activity[49,52]. The biological function of Ang(1-9) is to increase nitric oxide formation and release of arachidonic acid, enhance bradykinin activity[50] and possibly be involved in the inhibition of platelet function[53]. Ang(1-9) may decrease BP and thus protect the heart and blood vessels and reduce hypertension[54]. Ang(1-9) could mediate its actions *via* the AT2 receptors[54,55].

Angiotensin (1-7) [Ang(1-7)] was originally believed to be an inactive component of RAS. In 1988 this heptapeptide was shown to have actions opposing those of Ang Ⅱ[37]. Ang(1-7) is generated from Ang Ⅱ by ACE2 or by other known peptidases such as prolylendopeptidase and prolyl-carboxipeptidase[23,37,42,56]. Ang(1-7) can also be synthesized directly from AngI by prolylendopeptidase and from Ang(1-9) or from prohormone Ang(1-12) bypassing the synthesis of Ang Ⅱ[37,56]. Furthermore, Ang(1-7) interacts with the kallikrein-kinin system, and can be converted into Ang(1-5) or into Ang (3-7)[22]. Ang(1-7) levels are elevated by ACE inhibitors that increase AngI concentration and on the other hand prevent Ang(1-7) degradation[37].

Ang(1-7) was thought to be devoid of biological functions[37]. Nowadays Ang(1-7) is seen as a protector peptide that counterbalances many functions of Ang Ⅱ by binding to MasR which mediates vasodilating and antiproliferative functions of Ang(1-7)[23,36,55,57]. Although MasR is the main receptor of Ang(1-7), some of the functions may still originate *via* AT1R and AT2R[54,55,57,58]. In addition to the inhibition of Ang Ⅱ-induced vasoconstriction by Ang(1-7), its antiarrhythmogenic, antithrombogenic and growth-inhibitory properties suggest that Ang(1-7) acts as a physiological counterregulator within the RAS, and that Ang(1-7) could be a potential target for drug development[33-35]. In fact, Ang(1-7) has been associated to pathophysiology of several diseases such as. hypertension[59-63], chronic renal diseases[61] and diabetic nephropathy[64,65].

In addition to previously described peptides, RAS cascade includes short peptides which functions and roles in this circulating and tissue-specific regulatory system are still poorly known.

Key enzymes of RAS

Renin, ACE1 and ACE2 are seen as three key enzymes of the RAS. Renin, a specific enzyme having only one known substrate, is an aspartyl protease that cleaves its substrate angiotensinogen to form Ang Ⅰ. Renin cleaves the peptide bond between Leu10 and Val11 at the amino terminus of angiotensinogen. Renin is synthesized as a 406 amino acid residues long inactive prorenin in the juxtaglomerular apparatus of the kidney[22,36,37]. Upon demand synthesized prorenin is cleaved and activated by proconvertase or cathepsin B to generate 340 amino acid residues long catalytically active form of renin. Renin can also be synthesized in organs such as brain, heart, testis, pituitary and adrenal glands, arterial smooth muscle and eye[36]. Classically, renin is secreted by juxtaglomerular cells in response to three different stimuli: (1) decreased arterial BP; (2) decreased sodium levels in the macula densa ultrafiltrate; and (3) increased sympathetic nervous system activity[40,66,67]. Activation of prorenin can be either proteolytic or non-proteolytic. The proteolytic way is irreversible while the latter one is reversible[36].

ACE1 belongs to the M2 family of metallopeptidases containing zinc in its active site. ACE1 is a monomeric glycoprotein that has two different isoforms: somatic ACE1 (sACE1, 150-180 kDa) and germinal ACE1 (gACE1, 90-110 kDa)[36]. The somatic ACE1 is found in various epithelial and endothelial cells[68] whereas germinal ACE1 in germinal cells in the testis[36]. ACE1 is a type I integral membrane protein that consists of hydrophilic C-terminal cytoplasmic domain, hydrophobic transmembrane

domain and a heavily glycosylated N-terminal ectodomain[36]. It is distributed in many tissues and is also found in biological fluids, *e.g.*, in plasma and cerebrospinal fluid[69-71].

ACE1 has an activated water molecule complexed to Zn^{2+} in its active sites[72]. In addition, ACE1 activity depends on the presence of chloride that enhances the binding of different substrates[73]. As an exopeptidase ACE1 cleaves dipeptides from the free C-terminus of Ang Ⅰ and of the hypotensive peptide bradykinin[36,40]. ACE1 can also generate AngⅢ and Ang(1-7) and then further degrade Ang(1-7) to inactive Ang(1-5). Moreover, ACE1 acts in kallikrain-kinin system cleaving bradykinin to inactive compounds[36,40,57]. Because ACE1 participates in regulation of BP and in development of cardiovascular diseases, it is one major target for pharmacotherapy[36].

ACE2, the first known human homologue to ACE1 (42% sequence identity), was cloned in 2000[36,42,48,68,74]. ACE2 was first shown to convert AngI to Ang(1-9)[48]. Later, ACE2 was found to hydrolyze AngⅡ into Ang(1-7) with much higher efficiency (approximately 400-fold) than the hydrolysis of AngI to Ang(1-9)[36,42,49,57,75]. ACE2 is a 805 amino acid residues long (120 kDa) type Ⅰ transmembrane glycoprotein that has been found in organs such as kidney, heart, lungs, liver and brain. ACE2 has a conserved zinc metallopeptidase consensus sequence His-Glu-X-X-His, in wich X stands for any amino acid (HEXXH) in its active site and its activity is regulated by chloride ions[36]. Contrary to ACE1, primarily dipeptidylcarboxypeptidase, ACE2 functions as a monocarboxypeptidase cleaving a single amino acid residue (Phe) from AngⅡ to generate Ang(1-7). Thus, it negatively regulates the activated RAS and ACE1 activity by degrading AngⅡ and increasing Ang(1-7) formation[36,74]. ACE2 is not blocked by conventional ACE inhibitors[58].

ACE2 together with Ang(1-7) and MasR have become the focus of recent research regarding RAS[42,58]. ACE2 is seen as the key player maintaining the balance between the two main pathways of RAS: ACE1-AngⅡ-AT1R and ACE2-Ang(1-7)-MasR[36]. Chronic and long lasting imbalance of these two enzymatic pathways may lead to pathophysiology of the renal, pulmonary, cardiovascular and central nervous system[76].

In addition to previously mentioned enzymes, there are several different peptidases and proteases that act on longer angiotensin peptides thus cleaving them into shorter peptides. For example, AngⅡ can be generated from Ang Ⅰ by four different enzymes: ACE1, CAGE, chymase and cathepsin G[43]. Alternative enzymes acting on different angiotensin peptides are shown in Figure 1.

Alternative pathways for angiotensin II biosynthesis

A number of studies have shown alternative pathways for AngⅡ generation[77-79] being important in physiological and pathophysiological conditions[41,80]. AngⅡ-forming enzymes can be divided into three categories: metallodipeptidyl carboxypeptidase known as ACE1, aprotinin-sensitive serine proteases such as tonin[81], cathepsin G[82], kallikrein[83], trypsin[84] and chymostatin-sensitive serine proteases such as human chymase[85,86] (Figure 1).

Main receptors of RAS

Human (pro)renin receptor [(P)RR] is a 350 amino acid residues long single transmembrane-domain protein containing unglycosylated N-terminal domain responsible for renin and prorenin binding and the short cytoplasmic tail that is involved in the intracellular signalling[36,87]. Compared to the binding of free renin, the binding of renin to (P)RR is 3- to 5-fold more catalytically efficient, thus cleaving AGT to AngI more effectively[36,37].

Four heptahelical G-protein-coupled receptors of RAS: AT1R, AT2R, AT4R and MasR, mediate the effects of angiotensins causing vasodilatation and vasoconstriction[55,88]. AT1 and AT2 receptors are mainly responsible for mediating the effects of AngⅡ, whereas AT4 receptor is target of AngⅣ generated by degradation of AngⅡ[23,37]. A break-down product of Ang(1-7), namely Ang(3-7), can also bind to AT4R. AT4 receptors are located in the brain, lungs, heart, kidneys and liver and they are related to cognitive functions and proliferative effects[43,45,46].

Although AT1 and AT2 subtypes bind Ang Ⅱ in a similar manner, they differ in tissue-specific expression and genomic structure (only about 30% sequence homology) as well as in localization and regulation. AT1 receptors can be activated by Ang Ⅱ but other peptides, such as Ang Ⅲ, Ang Ⅳ and Ang(1-7), can also stimulate AT1R but with lower binding affinity[43]. AT1 and AT2 receptors mediate opposite effects of Ang Ⅱ, the former having negative cardiovascular effects, such as vasoconstriction and aldosterone release, and the latter having positive cardiovascular effects[12]. Whereas the role and function of AT1R is quite well established, the function of AT2R is not as clearly defined[55]. AT2 receptors, which are activated by Ang Ⅱ and also by Ang(1-7), may exert the antiproliferative, proapoptotic, vasodilatory and antihypertensive effects[43,89]. AT2 receptors are known to be involved in differentiation, regulation of growth and regeneration of neuronal tissue, and they are also known to play an important role in prenatal development. AT2 receptors can also inhibit AT1R signaling by directly binding into it. Thus they are considered to be cardiovascular protective receptors[12].

MasR was first discovered in year 1986 by Young *et al*[90] as proto-oncogene. Two years later high MasR levels were reported in the rat central nervous system by the same research group[91]. Later Kitaoka *et al*[92] described MasR expression in the eyes of rhesus macaque. It was early found in the mouse kidney and described as a factor involved in tumorigenesis[93]. Subsequently it is also found in other organs such as in heart, vessels, testis, kidney and brain[94] and very recently in the

human eye[95]. MasR is a G protein coupled receptor that has seven transmembrane domains[93]. This receptor acts antagonistically to the AT1R, mediating number of positive cardiovascular effects, such as vasodilation and antiproliferative effects, of its ligand Ang(1-7)[43]. MasR is part of the counterregulatory arm of RAS (ACE2-Ang(1-7)-MasR) thus balancing the effects of ACE1-Ang Ⅱ-AT1R pathway[34,35].

Tissue RAS

In addition to circulatory RAS, various organs have their own local RA-systems accounting for long-term changes and local effects including proliferation, growth and protein synthesis at tissue level[12,23,41]. The first clues of the existence of local RA-systems came in 1971 when Ganten *et al*[96] demonstrated that RAS components could be produced locally in organs and tissues. This proves that RAS is not only a circulating hormonal system, as thought earlier, but also a tissue-specific regulatory system[23]. Heart, liver, brain, kidney, lungs, intestine and even the human eye have their own local RA-systems[2,12,37].

Local RAS includes all components necessary for independent production of different components of RAS, such as Ang Ⅱ, angiotensinogen, ACE1, AT1R and AT2R[2,12,37]. Thus, RAS is not only an endocrine and circulating, but also a local paracrine and intracrine system regulating more functions than was previously thought[12,41]. Even though many of the local RA-systems operate independently from the circulatory RAS, in heart and kidney, tissue-RAS operates in close interaction with the systemic RAS thus complementing each other's functions[37]. Based on the origin of Ang Ⅱ, local RAS can be divided into extrinsic and intrinsic system, the former getting its Ang Ⅱ from the circulation and the latter obtaining its Ang Ⅱ through local biosynthesis[18].

LOCAL OCULAR RAS

RAS expression

Local RAS has also been identified in the human eye. Researchers have localized all of the central components of RAS, including its receptors, to the structures of the eye in variety of species[2,5]. Moreover, all components of the two main axes of RAS: Ang Ⅱ-ACE1-AT1R and Ang(1-7)-ACE2-MasR have been identified in the ocular structures of different species. When human eye is considered, the components of the two main axes are found in retinal structures and in non-retinal structures of the human eye[2,95,97]. Our research group has very recently succeeded to determine Ang (1-7) and ACE2 in the human aqueous humor[97]. Tables 1 and 2 summarize the localization of RAS peptides and enzymes in non-retinal ocular structures of the human eye. Tables 3 and 4 summarize the localization of RAS receptors in non-retinal ocular structures of the human eye. Although, essential components of RAS haven been identified in the human eye, the importance and functions of intraocular RAS are still unknown. However, intraocular RAS has been the focus of growing interest in recent years due to its possible role in the regulation of IOP through its effects on aqueous humor formation and drainage[5,12]. Furthermore, intraocular RAS activity has been linked to the development of glaucoma through its effect on IOP[2].

Concerning intraocular local RAS, there has been debate whether intraocular angiotensins originate from local production or from the blood compartment[14]. It has been shown that neither Ang Ⅰ, Ang Ⅱ nor angiotensinogen are able to pass the blood-brain barrier which is similar to blood-retina barrier in the eye[14,119,120]. Circulating angiotensins cannot reach the vitreous fluid when blood-retina barrier is intact[14]. However, if disrupted their entering the eye through blood-retina barrier becomes possible[99]. In porcine ocular tissues Ang Ⅰ and Ang Ⅱ levels are 5 to 100-fold over those found from admixture with blood or diffusion from blood[14]. In rabbit and pig ACE1 activity has been shown to be higher in ocular tissues than in plasma[121,122]. The local intraocular RAS is estimated to have a role in the regulation of IOP affecting the formation of aqueous humor and the drainage. It has been shown that systemic antihypertensive RAS-inhibiting medications lower IOP. Certain ACE inhibitors[15] and AT1 receptor blockers[16] have proved to lower IOP in both non-glaucomatous and glaucomatous patients. In animal studies, ACE inhibitors[17,18], AT1 receptor blockers[19,20] and renin inhibitors[21] have been reported to reduce IOP. It has also been suggested that Ang Ⅱ can increase aqueous humor secretion *via* AT1 receptor[118].

Aqueous humour dynamics and IOP

Aqueous humor formation: Intraocular pressure (IOP) can be described as a net sum of homeostatic balance between aqueous humor formation and outflow[123,124]. In the healthy human eye, the flow of aqueous humor against the resistance generates an IOP of about 15 mmHg[125]. Maintaining the optimal physiological IOP is fundamental to keep the optical and refractive properties of the eye, including the right shape of the eye[124,126]. The circulating fluid nourishes unvascularized eye structures such as the cornea and the lens. The normal aqueous humor formation rate is 2.5-2.8 μL/min and the entire volume is replaced every 100 min[5]. This is reduced during sleep, with ageing, and in some systemic diseases like diabetes[127]. Currently IOP is the main risk factor for glaucoma that is amenable to treatment[128].

The ciliary body epithelial is responsible for the production of aqueous humor[123] which is secreted mainly by active ionic transport across the epithelium against a concentration gradient[129]. Active secretion requires energy, produced in hydrolysis of adenosine triphosphate (ATP) by Na^+/K^+ ATPase. Active transport of Na^+ into the posterior chamber by the non-pigmented ciliary epithelial cells induces also water movement from the stromal pool into the posterior chamber. Active transport of Cl^- and HCO_3^- occurs to a lesser extent[130]. In addition to the active secretion two other physiological

Table 1 Renin-angiotensin system components in tears, lacrimal gland, bulbar conjunctiva, cornea, trabecular meshwork, aqueous humor and iris

RAS component	Tears lacrimal gland	Bulbar conjunctiva	Cornea	Trabecular meshwork	Aqueous humor	Iris
Prorenin		White *et al*[98]	White *et al*[98]		Danser *et al*[99]	White *et al*[98]
Renin		White *et al*[98]	White *et al*[98]			White *et al*[98]
AGT		White *et al*[98]	White *et al*[98]		Chowdhury *et al*[100]	White *et al*[98]
ACE1	Vita *et al*[101]	Savaskan *et al*[13]	Savaskan *et al*[13]	Savaskan *et al*[13]	Vita *et al*[101]	Ferrari-Dileo *et al*[106]
	Sharma *et al*[102]				Weinreb *et al*[104]	
	Immonen *et al*[103]	White *et al*[98]	White *et al*[98]		Aydin *et al*[105]	White *et al*[98]
					Holappa *et al*[97]	
ACE2					Holappa *et al*[97]	
Ang I					Danser *et al*[14]	Danser *et al*[14]
						Osusky *et al*[107]
Ang II		Savaskan *et al*[13]	Savaskan *et al*[13]	Osusky *et al*[107]	Danser *et al*[14]	Danser *et al*[14]
				Savaskan *et al*[13]	Osusky *et al*[107]	Senanayake *et al*[108]
Ang(1–7)				Vaajanen *et al*[95]	Holappa *et al*[97]	

Table modified and updated from the table published by Giese et Speth, 2014. ACE1, -2: Angiotensin converting enzyme 1, -2; AGT: Angiotensinogen; Ang Ⅰ, -Ⅱ: Angiotensin Ⅰ, -Ⅱ; Ang(1–7): Angiotensin (1–7); RAS: Renin-angiotensin system.

Table 2 Renin-angiotensin system components in ciliary body, non-pigmented ciliary epithelium, lens, vitreous, optic nerve head and sclera

RAS component	Ciliary body/non-pigmented ciliary epithelium		Lens	Vitreous	Optic nerve head	Sclera
Prorenin	Sramek *et al*[109]	White *et al*[98]		Danser *et al*[99]		White *et al*[98]
	Danser *et al*[99]			Wallow *et al*[110]		
	Wallow *et al*[110]					
	Berka *et al*[111]					
Renin	Berka *et al*[111]	White *et al*[98]				White *et al*[98]
AGT	Sramek *et al*[112]			Sramek *et al*[112]		
ACE1	Igic *et al*[113]	Savaskan *et al*[13]		Ferrari-Dileo *et al*[106]	Ferrari-Dileo *et al*[106]	White *et al*[98]
	Ferrari-Dileo *et al*[106]	White *et al*[98]		Nakanishi *et al*[114]		
	Sramek *et al*[112]			Ishizaki *et al*[115]		
				Aydin *et al*[105]		
ACE2						
Ang I	Danser *et al*[14]					
Ang II	Danser *et al*[14]	Senanayake *et al*[108]		Senanayake *et al*[108]	Savaskan *et al*[13]	
	Savaskan *et al*[13]					
Ang(1–7)	Vaajanen *et al*[95]	Vaajanen *et al*[95]				

Table modified and updated from the table published by Giese et Speth, 2014. ACE1, -2: Angiotensin converting enzyme 1, -2; AGT: Angiotensinogen; Ang Ⅰ, -Ⅱ: Angiotensin Ⅰ, -Ⅱ; Ang(1–7): Angiotensin (1–7); RAS: Renin-angiotensin system.

processes exist in the fluid formation: diffusion from the blood compartment and ultrafiltration. They are passive and require no cellular activity[131]. The whole ciliary body system and its aqueous humor formation should be regarded as a multifunctional and interactive process. Aqueous humor is a mixture of organic solutes, electrolytes, growth factors, cytokines and proteins[132-136]. After the production it is secreted into the posterior chamber from where it flows between the lens and iris into the anterior chamber[132,137,138].

Aqueous humor outflow: *Via* anterior chamber and through the trabecular meshwork and the canal of Schlemm, aqueous humor escapes the eye into the venous blood system[123]. It can leave the eye through three different main routes: the trabecular, the uveoscleral or the uveolymphatic pathways[128]. Trabecular outflow is the main route of drainage accounting for 90% of all aqueous humor outflow, and it is pressure-dependent[5,128,139]. The fluid outflow through the trabecular meshwork is affected by adhesions of trabecular meshwork cells and by the state of the actin cytoskeleton[140].

Outflow, where aqueous humor drains through the ciliary muscle and exits through the supraciliary space and across the anterior or posterior sclera into choroidal vessels, is called the uveoscleral outflow[141] which is independent of IOP and particularly impacted by age[139]. A third outflow route is suggested to exist: channels in the stroma of the ciliary body and interstitial spaces between ciliary muscle bundles. It may function as a backup outflow system[142]. The relevance of this pathway remains to be determined. The other alternative, minor outflow pathways are *via* iris vessels, corneal endothelium, or anterior vitreous body[143].

Pharmacological treatment of glaucoma reduces IOP

Table 3 Renin-angiotensin system receptors in tears, lacrimal gland, bulbar conjunctiva, cornea, trabecular meshwork, aqueous humor and iris

RAS component	Tears lacrimal gland	Bulbar conjunctiva	Cornea	Trabecular meshwork	Aqueous humor	Iris
(P)RR		White *et al*[98]	White *et al*[98]			White *et al*[98]
AT, unknown subtype						Lin *et al*[116]
AT1R						Senanayake *et al*[108]
AT2R						Senanayake *et al*[108]
AT4R						
MasR			Vaajanen *et al*[95]	Vaajanen *et al*[95]		

Table modified and updated from the table published by Giese et Speth, 2014. AT1, 2, 4: Angiotensin Ⅱ type 1, 2, 4 receptor; MasR: Mas receptor; (P)RR: (pro)renin receptor; RAS: Renin-angiotensin system.

Table 4 Renin-angiotensin system receptors in ciliary body, non-pigmented ciliary epithelium, lens, vitreous, optic nerve head and sclera

RAS component	Ciliary body/non-pigmented ciliary epithelium		Lens	Vitreous	Optic nerve head	Sclera
(P)RR	White *et al*[98]					White *et al*[98]
AT, unknown subtype	Lograno *et al*[117] Lin *et al*[116]					
AT1R	Cullinane *et al*[118]	Senanayake *et al*[108]			Senanayake *et al*[108]	
AT2R		Senanayake *et al*[108]			Senanayake *et al*[108]	
AT4R						
MasR		Vaajanen *et al*[95]				

Table modified and updated from the table published by Giese et Speth, 2014. AT1, 2, 4: Angiotensin Ⅱ type 1, 2, 4 receptor; MasR: Mas receptor; (P)RR: (pro)renin receptor; RAS: Renin-angiotensin system.

by decreasing the rate of aqueous humor formation or by increasing the rate of aqueous humor outflow[144].

Glaucoma

It is well-known that defects in the RAS cascade are involved in several cardiovascular and renal diseases, including heart failure, hypertension, ventricular hypertrophy, cardiac remodelling, and chronic renal failure[145-147], but interestingly, imbalances in the RAS cascade are also involved in glaucoma[3], which is a neurodegenerative disorder that leads to the loss of the axons populating the optic nerve and to the death of retinal ganglion cells by non-apoptotic and apoptotic mechanisms[2,3,6]. Together with age and family history, increased IOP is one of the known major risk factors for glaucoma[2,6,7]. Diabetes, migraine/vasospasms and vascular dysfunction are also considered as risk factors for glaucoma development[5,6,128].

Ocular hypotensive medications, laser procedures and surgical means are currently the major therapeutic tools to treat glaucoma[2,6,22]. They all act by lowering IOP thus affecting the onset of the disease[5]. Interestingly, antihypertensive medications acting on RAS have been shown to lower also IOP, suggesting that compounds blocking RAS might be potential anti-glaucomatous drugs in the future[22]. ACE inhibitors can decrease Ang Ⅱ levels in aqueous humor[107]. By reducing blood flow in the ciliary body ACE inhibitors could also decrease aqueous humour production[148]. Furthermore, by preventing the breakdown of bradykinin ACE inhibitors are able to promote synthesis of endogenous prostaglandins, which, as shown with marketed prostaglandin analogues, could increase the uveoscleral outflow thus lowering IOP[149,150]. Biosynthesis of certain matrix metalloproteinases is thought to be associated with increased uveoscleral outflow which leads to relaxation of the ciliary muscle and reduction and compaction of extracellular matrix components within the ciliary muscle, the sclera, the iris and within tissues of the uveoscleral outflow route, all of which might lower IOP by facilitating aqueous humor outflow[151]. ACE-inhibitors activate also the nitric oxide pathway by preventing bradykinin breakdown which increases endothelial nitric oxide formation and causes vasodilatation. Bradykinin stimulates the synthesis of prostaglandins and nitric oxide which also antagonize the vasoconstrictive effects of endothelin-1 and inhibit the overall production of endothelin-1 by endothelial cells. Endothelin 1 is a vasoconstrictive peptide that promotes contraction in the human ophthalmic artery and in the porcine ophthalmic and ciliary arteries[152-154].

Moreover, RAS activity has been described in cultured non-pigmented human ciliary epithelial cells which participate in aqueous humor formation and many of the central components of RAS have been identified in eye structures responsible for aqueous humor formation such as ciliary body[2,116,118]. Ang Ⅱ can activate Ca^{2+} signalling system that increases potassium ion channel activity[155]. Together with cell volume loss, these effects suggest that Ang Ⅱ acts as a operated secretagogue in the non-pigmented ciliary cells[118]. In

addition, Ang Ⅱ activates Na^+/H^+ exchange which leads to an increase in cytoplasmic sodium concentration[129]. In ciliary and renal tubular epithelium sodium handling related mechanisms are common pathogenetic factors. This might explain the coexistence of glaucoma and systemic hypertension[156]. Other explanations have also been suggested for the relationship between hypertension and glaucoma development. Hypertension is shown to cause impairment in autoregulation of the posterior ciliary circulation[157] and suggested to induce microvascular damage thus worsening blood flow to the optic nerve[158]. Furthermore, antihypertensive therapy has been described to cause hypotensive episodes that can injure the optic nerve[159].

In addition to possible role of RAS in the aqueous humor formation, RAS is suggested to act in aqueous humor outflow. Ang Ⅱ is able to promote cell proliferation in bovine trabecular meshwork cells and increase synthesis of collagen *in vitro*. Moreover, intracamerally administered Ang Ⅱ reduces uveoscleral outflow[160]. Paradoxically, natural and synthetic Ang Ⅱ, when administered intravenously, lowered IOP in anaesthetized cats[161].

RAS AND OTHER EYE DISEASES

In addition to glaucoma, local intraocular RAS has been associated with other severe eye diseases that can lead to permanent vision loss, such as age-related macular degeneration (AMD), ROP and DR. Dysregulation of RAS cascade participate in the development of these severe eye diseases.

AMD

In elderly people, AMD is one of the leading causes of visual impairment. Both dry and wet forms of the disease are associated with vision loss. Dry forms of the disease accounting for 90% of the cases lead to the significant decline of photoreceptors which ultimately causes central vision loss. On the contrary, wet form of AMD is characterized with pathological growth of cloroidal blood vessels that will eventually populate retina after breaking through the underlying Bruch's membrane. In addition to old age, environmental factors, smoking, genetic susceptibility and systemic hypertension are regarded as risk factors for developing AMD. Interestingly dysregulation of the RAS cascade is suggested to play a role in the development of AMD[2,162,163].

Three key observations are held as evidence showing the possible involvement of RAS in the development of AMD. Firstly, systemic hypertension is a risk for the development of AMD. Secondly, dysregulation of RAS may have an impact on retinal pigment epithelium function and photoreceptor viability due to the observations that Ang Ⅱ can modulate retinal pigment epithelium. Thirdly, Ang Ⅱ is involved in retinal angiogenesis thus it might have a role in choroidal neovascularisation[2,162]. Animal studies have proven that administered AT1R antagonist (losartan)[164] and other AT1 receptor blockers[165] and (pro)renin receptor inhibitor[166] can reduce choroidal neovascularization thus having a positive effect on AMD.

ROP

ROP is a neovascular disease affecting premature newborns. ROP is associated with pathological retinal neovascularisation that causes complications such as tractional retinal detachment, macula dragging and vitreal haemorrhage, all of which can lead to vision loss[162]. The main risk factors for the disease are low birth weight and lower gestational age, both of which correlate with immaturity of retina at birth. In fact, in industrialized countries, approximately two-thirds of infants with birth weight less than 1.25 kg manifest some degree of retinopathy[167]. The cause of ROP is thought to be the retinal blood vessels expanding from the optic nerve which growth halts when a premature neonate is brought into a high oxygen environment. When the newborn is brought back to normal conditions, the inner vasculature in retina fails to regain normal vessel growth thus creating an avascular area and causing neovascularisation and epiretinal angiogenesis that can lead to vision loss[168].

Studies using animal models have suggested that RAS is involved in the development of ROP. Infants that are diagnosed with ROP have had elevated serum prorenin levels[169], ocular renin levels[170,171] and increased AT1R and AT2R expression[170]. Treating oxygen induced retinopathy in animal models with ACE inhibitors and AT1R antagonists during the normal air conditions reduces pathological angiogenesis on the surface of the retina[170,172-174]. On the contrary, the role of AT2R in retinal vascular pathology and the effects of the use of AT2R antagonists on retinal angiogenesis are still debatable[171,173,175,176].

Diabetic retinopathy

The development of progressive vascular pathology within the inner retina characterizes DR which is among of the leading causes of blindness worldwide[163,177]. Alterations in the blood-retinal barrier, ischemia, dilated capillaries associated with poor retinal perfusion, retinal microaneurysms, loss of pericytes leading to changes in vascular permeability and the release of growth factors which may induce neovascularisation are all implications of DR[178]. DR can occur as non-proliferative DR (NPDR), which corresponds to the early state of the disease, or as more advanced form of the disease: proliferative DR (PDR). In NPDR the breakdown of the blood-retinal barrier and weakened retinal blood vessels lead to the formation of microaneurysms that can leak fluid into retina causing swelling of the macula. In PDR blood vessels can grow into the vitreous and on the surface of the retina[177,179]. Blocking the RAS cascade seems to reduce the incidence and progression of DR suggesting that RAS may be implicated in the pathogenesis of the disease[180-182]. However, more research is required to understand the complex interplay between RAS cascade and DR.

CONCLUSION

Systemic RAS regulates BP homeostasis, body fluid volume and electrolyte balance. An interesting new observation is intraocular, local RAS, especially existed in the eye structures which are involved in aqueous humor dynamics. Human and animal studies have both shown that antihypertensive drugs blocking RAS at any level can reduce IOP suggesting that these kind of compounds may be potential anti-glaucomatous drugs in the future. Furthermore, compounds elevating Ang(1-7) formation, activating Mas receptors and positively affecting ACE2 activity offer new intriguing opportunities for ocular pharmacology in the future. Although IOP represents the major risk factor in glaucoma, reduction of IOP does not always prevent the progression of disease like in low-tension glaucoma, indicating that factors other than elevated IOP are involved in glaucoma progression. Apoptosis of retinal ganglion cells may be the main possible unsolved reason. ACE inhibitors[183], ARBs[184] and Mas-receptor ligands[185] have showed some potential neuroprotective effects, which will stimulate research activity in the future.

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MINIREVIEWS

Intravitreal drug administration for treatment of noninfectious uveitis

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Abstract

Intravitreal treatment became popular with the discovery of the blood ocular barriers, which significantly limit drug penetration in systemic or topical administration. As the mainstay of treatment in noninfectious uveitis (NOIU) is still corticosteroids, triamcinolone acetonide (TA) was the first intravitreally used agent in this subset of patients. Although it was very effective in controlling inflammation and improving the inflammation related complications, TA was found to have a high rate of intraocular complications and a relatively short half-life necessitating frequent reinjections. Other systemically used therapeutic options such as methotrexate and anti-tumor necrosis factor-α agents were also tried intravitreally. Additionally anti-vascular endothelial growth factor agents that are widely used intravitreally in the management of diabetic retinopathy and age related macular degeneration have become an option to control the uveitis related complications like macular edema, retinal and choroidal neovascularizations. Advances in biotechnology led to the slow release biodegradable implant era. These implants have a longer duration of action, which may help in decreasing the number of reinjections. Today two forms of implants have been approved for use in NOIU, Retisert (0.59 mg flucinolone acetonide, surgical intervention) and Ozurdex (0.7 mg dexamethasone, office based intervention). Studies dealing with newer agents (cyclosporine, LFG31, sirolimus) in the management of chronic NOIU are on the way. The search for ideal effective, safe and biocompatible intravitreal agents in the management of NOIU has not ended yet.

Key words: Uveitis; Intravitreal; Steroid; Implant

Core tip: The limitations related to the systemic use of treatment options in noninfectious posterior uveitis yielded intravitreal route. The hallmark of intravitreal treatment triamcinolone acetonide has a short half-life with a high rate of intraocular complications, and this led to the development of implants as a treatment option with various agents in the market still under

investigation. In this review, we try to summarize the intravitreal therapeutic options that are being used in noninfectious uveitis.

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INTRODUCTION

Ohm first described the use of intravitreal (IV) injections for therapeutic purposes in 1911 with injection of air in the repair of retinal detachment[1]. The therapeutic use of the IV route was not developed until the early 1970s, when investigations about the blood ocular barriers were started. The results of these investigations increased the use of the IV route which enables us to bypass anatomical barriers, for the administration of therapeutic agents[1]. From the middle of the 20th century, several agents such as antibiotics, antivirals, antifungals, steroids, anti-vascular endothelial growth factors (anti-VEGFs), immunomodulatory, anti-inflammatory, and antineoplastic agents have been used intravitreally[2-6]. Nowadays, as a method for providing higher therapeutic levels especially in the posterior segment of the eye, the IV route is widely used in many blinding diseases such as age related macular degeneration, diabetic retinopathy, vascular occlusions, macular edema, endophthalmitis, viral retinitis and ocular inflammatory disorders.

Noninfectious uveitis (NOIU) with posterior segment involvement is one of the ocular diseases in which IV injection is required. The mainstay of treatment in this subset of disease and its sight-threatening complications is still systemic corticosteroids. However, to overcome the blood ocular barrier effect, higher doses are needed causing higher risk of systemic side effects like hypertension, osteoporosis, diabetes mellitus, gastritis, skin thinning, hyperlipidemia and many fluid-electrolyte imbalances[7,8]. It is also important to note that children are more prone to side effects related to corticosteroids such as growth retardation, precocious puberty, immune and hypothalamic-pituitary-adrenal axis suppression[8]. Second line treatment, used for steroid sparing, consists of immunosuppressive and immunomodulatory agents, but these too have a serious systemic side effect profile. Thus, local therapy remains an attractive treatment of choice especially in uveitis that is not associated with systemic diseases, in unilateral presentation, and in patients with compliance problems for systemic drug use. It also offers an excellent adjunctive therapeutic opportunity in cases where adequate control of inflammation cannot be provided despite systemic treatment. As the blood ocular barriers do not permit topical treatment to achieve a sufficient therapeutic level in the posterior segment, local treatment by IV route serves as a good solution in posterior segment uveitis. IV triamcinolone acetonide (IVTA) has been the most widely preferred option but has a short half-life and limited duration of action. It also has important ocular side effects like cataract and glaucoma, which mostly require surgical intervention[9,10]. The evolution of IV injections has led to the development of IV implants which aim to increase the duration of action and decrease the number of injections.

In this paper we aim to perform a literature review of recent developments in IV treatment of NOIU.

CORTICOSTEROIDS

Triamcinolone acetonide

IVTA is effective in controlling vitritis, reducing macular edema and improving visual acuity with IV doses of 2 to 4 mg when applied in NOIU with posterior segment involvement[11-13]. Its method of action is *via* different pathways including the inhibition of phospholipase A synthesis, blocking the production of inflammatory cytokines, stabilizing the blood retinal barrier and reducing VEGF levels[5,14]. Kramer *et al*[15] found that IVTA was very effective in rapid clearing of the vitreous inflammation with improvement in the visual acuity when used either alone or in combination with systemic immunosuppressive therapy. Lasave *et al*[5] used a single IVTA injection in refractory uveitic cystoid macular edema and reported that both visual acuity and macular thickness measurements had improved successfully at the 6th month visit. They also found that there was a significantly better visual improvement in macular edema cases with duration of less than a year, and therefore suggested earlier use of IVTA in refractory cases. A similar efficiency was reported by Karacorlu *et al*[16] who also found that IVTA achieved an improvement in visual acuity at the end of 6-mo follow-up in 30% of cystoid macular edema cases due to Behcet's disease. Angunawela *et al*[17] published their long-term results of IVTA injections in uveitic macular edema refractory to systemic and orbital floor steroid injections and concluded that IVTA is effective. They stated that although retreatment is required, this can be maintained with orbital floor injections. In their series, 9 of the 12 eyes had increased visual acuity at the final control (mean 40.5-mo follow-up) while 3 of them were resistant.

One of the main limitations of the IVTA is the off-label use in Europe and many other countries and the preservative used which might be toxic to the retina. The second limitation is its relatively short duration of action lasting approximately 3-7 mo that necessitates frequent re-injections[18]. It is important to note that the vitreous half-life of IVTA in vitrectomized eyes is shorter since the clearance is quicker[10,19,20]. The third and most important limitation is the occurrence of ocular side effects such as cataract and intraocular pressure elevations. Approximately 1%-2% of cases require

Table 1 Summary of some intravitreal agents

	Application	Duration of action	Visual acuity	Glaucoma surgery	Cataract surgery
IVTA 4 mg (kenalog)	Injection	3-7 mo[17]	58.3% gained ⩾ 2 Snellen lines with a median 40.5-mo follow-up[16]	1%-2%[10]	15%-30%[10]
FA 0.59 mg (retisert)	Surgical implant	30 mo[21]	23% gained ⩾ 3 lines after 3 yr[21]	32%-40%[21,23,25]	Nearly 100%[21,23,25]
Dexamethasone 0.7 mg (ozurdex)	Non-surgical implant	4-6 mo[21]	38% gained ⩾ 3 lines at 6th month[29]	None[30]	1.3%[30]
MTX 400 μg	Injection	4 mo[21]	38% gained ⩾ 2 lines at 3rd month[21]	None[21]	None[21]

IVTA: Intravitreal triamcinolone acetonide; FA: Flucinolone acetonide.

glaucoma surgery, 15%-30% require cataract surgery, and the risk of the need for these procedures increases with the number of reinjections[11].

Both frequent reinjection necessity and a high risk of intraocular complications have driven researchers to investigate long-lasting implantable IV agents with different glucocorticoid agents. Nowadays, flucinolone acetonide (FA) (Retisert, surgically implanted) and dexamethasone (Ozurdex, non-surgically implanted) implants are being used in NOIU and considerable data with regards to their efficiency and side-effect profile have been collected.

FA

The beneficial effect of surgically introduced IV implant of ganciclovir for the treatment of cytomegalovirus retinitis is the hallmark in development of the posterior segment implants. This route seems to be a perfect solution for chronic NOIU with a probable improvement in the duration of action, which is the major limitation of IVTA. FA with its low water solubility is the first Food and Drug Administration (FDA) approved glucocorticoid implant (Retisert, Bausch and Lomb, Rochester, NY) to be used in NOIU[21]. The implant is surgically placed and contains 0.59 mg FA that is slowly released up to 30 mo allowing the opportunity of tapering systemic medications, avoidance of multiple IV injections and possible concurrent complications of injections. The comparison of eyes, one having implant and the other not, revealed that the FA implant reduced the recurrence rate significantly from 62% to 20% in the implanted eye whereas recurrence was 59% in non-implanted eye at the end of the 3-year follow-up[22,23]. In the Asian population, Sangwan *et al*[24] reported similar effectivity with a 0.59 mg dose to prevent recurrences with the rates declining from 43.6% to 17.1%. Studies have also found FA implant to be very successful in improving visual acuity and in reducing the need for adjunctive systemic or periocular steroid treatments[22,24,25]. Callanan *et al*[22] stated that the visual acuity increased ⩾ 3 lines in 23% of the 0.59 mg FA implanted eyes compared to 6% in non-implanted. The same rate was 31.1% *vs* 7.6% in Sangwan *et al*[24] study.

The major ocular side effects of the FA implant are cataracts and raised IOP. Nearly all of the patients required cataract surgery and 32%-40% required IOP lowering filtration surgery at the end of the 3-year follow-up[22,24,26]. Other ocular complications worthy of mention are retinal detachment (4.0%), endophthalmitis (1.0%), and hypotony which could occur at any time in 3-year follow-up (34.0%)[21]. Although 0.59 mg FA implant requires surgical implantation and further surgical interventions to treat ocular side effects like cataract and glaucoma, a recent review that compared systemic corticosteroid *vs* 0.59 mg FA implantation in terms of cost-effectivity has found the implant to be reasonably cost-effective in unilateral noninfectious intermediate, posterior and panuveitis cases[27].

Iluvien (Alimera Sciences Inc., Alpharetta, GA) is another FA implant approved to be used in diabetic macular edema. Its difference from Retisert is that Iluvien can be applied in the office setting without the need for surgical intervention. It also releases lower doses of medication and preliminary data suggest that the risk of a rise in IOP is lower compared to Retisert[28]. However, there are no data up to date for its use in uveitis.

Dexamethasone

Dexamethasone is approximately 3-5 times more potent compared to triamcinolone acetonide (TA) and 7.5-12.5 times more potent compared to FA. Its implant form is Ozurdex (Allergan Inc, Irvine Calif, United States) which is a bioerodible device composed of a mix of polylactic acid and polyglycolic acid polymers that releases 0.7 mg of dexamethasone for up to 6 mo. One of the major advantages over the former approved glucocorticoid implant Retisert is the office based application without any need for surgery[29]. The FDA approved its use in retinal vein occlusion, uveitis and diabetic macular edema[30]. The first data about the use of Ozurdex in uveitis were gathered from the results of HURON (Chronic uveitis evaluation of IV dexamethasone implant) trial[31]. The HURON study revealed that a single injection resulted in efficient control of inflammation and good visual outcomes for up to 6 mo in noninfectious intermediate or posterior uveitis. A recent multicenter study which evaluated Ozurdex implants in NOIU confirmed the success of the implant in controlling vitreous haze, cystoid macular edema and visual acuity[30]. Authors noted that the improvement in uveitis presentation can be observed as early as 2 to

4 wk after the injection. The percentage of eyes that gained ⩾ 3 lines in visual acuity were 38% at the end of the 6th month. The median time to reinjection was 10 mo and the time to uveitis relapse considering the changes in macular thickness, vitreous haze and visual acuity was 6 mo, which is comparable to the previously performed studies[32,33]. The main problems with the former glucocorticoid implant Retisert (high rate of a raised IOP and cataracts) were found to be significantly less with Ozurdex. The HURON study reported that only 23% of eyes required IOP lowering medications without any surgical intervention and 1.3% needed cataract extraction[31] (Table 1).

Zero point seven mg dexamethasone implant Ozurdex has many advantages, *i.e.*, 22G office based application and lower risk of IOP rise and cataract formation. However, considering the disease is mostly chronic and recurrent, reinjections are mostly needed.

Methotrexate

Methotrexate is an antimetabolite immunosuppressive that has been used in NOIU for many years as a steroid sparing agent[34,35]. It is also used in the treatment of intraocular lymphoma cases as IV injections at 400 μg doses[36,37]. In a retrospective study, Hardwig *et al*[38] reported that IV methotrexate preserved or improved visual acuity in seven of eight uveitis patients. Similarly, in a prospectively designed study Taylor *et al*[39] have announced that in 30 of 38 eyes, intraocular inflammation was successfully controlled with improved vision and without any ocular side effects. From 30 eyes that responded well, only 8 have relapsed and 7 of them responded to the reinjection. They also emphasized that 57% of the patients were able to reduce systemic treatments. IV methotrexate might serve as a preferable option in noninfectious posterior uveitis with high efficacy, nearly no side effect and an extended duration of action (Table 1).

Anti-tumor necrosis factor-α

Anti-tumor necrosis factor-α (TNF-α) is a pro-inflammatory cytokine that is involved in regulation of immune cells, tumor suppression and inhibition of viral replication[40,41]. It is also mentioned in the pathophysiology of ocular inflammatory conditions related to autoimmune diseases and ocular diseases that have an inflammatory component such as diabetic macular edema and neovascular age related macular degeneration[42-45]. There is a significant amount of data on systemic use of anti-TNF-α agents in uveitis especially in Behcet's disease, juvenile idiopathic arthritis and ankylosing spondylitis. However, the systemic side effects like fatal blood disorders, secondary infections, reactivation of latent infections, and demyelinating nerve system disorders limit its use[46]. As in the case of glucocorticoids, IV route was tried to avoid systemic side effects. For all TNF-α agents, the optimal IV dose was decided after the animal studies were completed. The results of the studies that will be discussed in this paper are mostly case series and the literature lacks standardized well-designed prospective works.

Etanercept was studied in a pilot study involving seven patients with resistant diabetic macular edema. At the end of 3 mo, no significant improvement or side effects were seen with a safe dose of 2.5 mg IV injection that was repeated at 2 weekly intervals[47]. It was then abandoned and no further studies were conducted afterwards. Thus, there are no available data on its use in uveitis.

Infliximab, a murine-based monoclonal antibody, was investigated in animal studies and IV doses below 2 mg were reported to be well-tolerated[48]. The Pan-American Collaborative Retina Study Group, the largest series that was conducted about the IV use of infliximab in diabetic macular edema and exudative age related macular disease, has concluded that IV infliximab did not result in any anatomic or functional benefit whereas 37.5%-42% of the injected eyes developed severe uveitis[49,50]. Its use in noninfectious posterior uveitis and Behcet's disease was found to improve vision initially but failed to stabilize the vision in the long-term[51,52]. In short, studies demonstrated that IV infliximab might be useful in uveitis but not in diabetic macular edema or exudative macular disease.

Adalimumab is also one of the preferred anti-TNF-α options that is successfully used in the treatment of NOIU[53]. Hamam *et al*[54] recently published the only study of IV adalimumab use in human. They performed an IV adalimumab injection of 0.03 mL (1.5 mg) at 0, 2 and then every 4 wk for a total 26-wk duration in 7 patients (13 eyes). Only 1 patient had worsened ocular inflammation and was removed from the study and switched to systemic and local corticosteroid treatment. Visual acuity improved in 7 of 12 eyes with ⩾ 2 ETDRS lines, whereas the other 5 eyes remained stable or improved 1 line. In 8 eyes with macular edema, 5 achieved complete resolution. No ocular or systemic side effects were reported. Authors had noticed that 4 patients had Behcet's disease, which might affect the results since anti-TNF-α has favorable results in this particular disease. More numerous studies are required to reach a conclusion about the IV use of adalimumab.

Anti-VEGF agents

IV anti-VEGF agents are widely used for age related macular degeneration related choroidal neovascularizations, and macular edema related to diabetic retinopathy and retinal vascular occlusions[55,56]. Their use in uveitis is mostly related to the management of secondary complications of uveitis such as macular edema and choroidal neovascularizations[57,58]. In a study comparing IV anti-VEGF agents and IVTA, Lasave *et al*[5] reported that a single injection of IVTA is superior to IV bevacizumab in chronic resistant uveitic macular edema cases with regards to improvement in visual acuity and macular thickness. A prospective non-comparative

therapeutic trial has been published recently evaluating the effect of ranibizumab on macular edema in clinically well-controlled 5 eyes of 5 uveitis patients. They performed 4.6 injections on average in the first 6 mo and 1.8 injections in the second 6-mo period according to the criteria they put forth at the beginning of their study. The 12th month follow-up visit for the same study revealed that there was a statistically significant 12.2 letter increase in visual acuity and 45.4% decrease in macular thickness. Another interesting study about the effect of anti-VEGF agents in uveitis was the retrospective study performed by Al-Dhibi *et al*[59] that evaluated the effect of bevacizumab in infectious uveitis and NOIU. Similarly, they reported improvement in visual acuity and macular thickness. The latest finding is that bevacizumab is effective and safe without any immunosuppressive effect against infectious agents.

In summary, they are not superior to IVTA and have short half-life necessitating reinjections. Therefore, they do not seem to be ideal agents for uveitis, which is mostly chronic and recurrent. The major advantage of these agents might be the relatively low incidence of ocular complications like cataract and IOP rise when compared to glucocorticoids. This might be very helpful especially in steroid responder cases. Additionally, they might be of use in uveitis induced choroidal or retinal neovascularizations.

Future intraocular devices and agents for the treatment of NOIU

I-vation is a screw shaped implant, which is twisted through the pars plana from a 0.5 mm sclerotomy. It contains 0.925 mcg TA that is reported to have 1-year duration of release. The 1-year results demonstrated that it was effective in diabetic macular edema with decrement in macular thickness and increment in visual acuity[60]. The phase 2 results have not been published yet. There are no data for uveitis patients as of yet.

Sirolimus, a macrolide antibiotic (rapamycin), was originally developed as an antifungal agent. After the immunosuppressive and antineoplastic effects were discovered, it is now being investigated for the treatment of different ocular diseases including uveitis. It suppresses T and B cell proliferation and inhibits interleukins-2, -4 and -5[61]. Sirolimus as Therapeutic Approach to Uveitis study has announced its 6-mo results, which reported equal success in improving vitreous haze with subconjunctival or IV administration[62]. The ongoing phases 2 and 3 studies will help clinicians to reach a better conclusion about the effectiveness and safety profile of local sirolimus treatment in NOIU.

LFG316 is a monoclonal antibody that inhibits activation of complement protein 5 and a phase 1 single ascending dose study of IV injections was performed in advanced AMD patients[63]. The IV use in multifocal choroiditis and panuveitis is currently under investigation.

Cyclosporine is a well-known second-line immunosuppressive agent, which is used especially in chronic NOIU patients. The IV implant form of cyclosporine was tested in 2 experimental uveitis models in rabbits and found to be effective and safe[64,65].

CONCLUSION

Uveitis is still one of the most challenging issues of ophthalmology from diagnosis to treatment. For a long time, corticosteroids served as the only treatment option in NOIU and are still the mainstay of treatment although many new agents have emerged. The IV route is a great option for clinicians to reach therapeutic levels in the posterior segment of the eye, since the blood ocular barriers significantly limit the efficacy of topical and systemic administrations. It also allows for a reduction in systemic treatment doses of therapeutic agents and thus a decrease in side effects related to higher doses. IV treatment is an excellent treatment of choice especially in cases with unilateral involvement, in uveitis not associated with systemic disease and in patients who have problems with systemic drug use. It is also a good adjunctive treatment in patients with active ocular inflammation despite optimal systemic therapy. The high rate of cataract, IOP rise and relatively short half-life, which requires frequent reinjections with conventional IVTA, has evoked the innovations of implant technology. Today, Retisert and Ozurdex are the most commonly preferred glucocorticoid options in uveitis management with some advantages and disadvantages. The systemic agents that are being successfully used in NOIU management (methotrexate, anti-TNF-α agents) are also being tested for IV administration. IV anti-VEGF agents might be an option for uveitic macular edema especially in steroid responder cases. However, studies performed for evaluation of IV drug administration in uveitis are mostly non-standardized (length of follow-up, doses, patient selection, criteria for effectiveness) and retrospective case series with small samples, which limit the clinicians' ability to reach a conclusion. It seems that the search for safe, cost-effective and long acting agents in uveitis management has not reached to an end yet.

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Diabetic macular edema: Efficacy and safety of anti-vascular endothelial growth factor therapy

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Abstract

Diabetic retinopathy is one of the prominent causes of vision impairment in the working-age population in industrialized countries and is related to 1%-5% of cases of blindness in the world. Among patients with diabetic retinopathy, diabetic macular edema (DME) is the major reason of vision impairment and represents a significant public health problem. Previous studies demonstrated the role of vascular endothelial growth factor (VEGF) in diabetic retinopathy and DME pathogenesis, and also revealed the efficacy of anti-VEGF agents for the management of these disorders. This review summarizes the outcomes of clinical studies that evaluated the anti-VEGF therapy including pegaptanib, ranibizumab, bevacizumab, and aflibercept for the management of DME. A significant number of clinical trials indicated favorable functional and anatomical results of anti-VEGF therapy for DME. Therefore, these agents should be considered an option in the treatment of DME in routine clinical practice.

Key words: Anti-vascular endothelial growth factor; Aflibercept; Bevacizumab; Diabetic macular edema; Pegabtanib; Ranibizumab

Core tip: Diabetic retinopathy is one of the prominent reasons of vision loss in the industrial countries. Among these patients, diabetic macular edema (DME) is the main reason of vision impairment. Previous studies have shown that vascular endothelial growth factor (VEGF) has a major role in the pathogenesis of diabetic retinopathy and DME, as well as demonstrated favorable results for DME treatment. This review summarizes the outcomes of clinical trials that evaluated anti-VEGF agents including pegaptanib, ranibizumab, bevacizumab, and aflibercept in DME treatment.

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INTRODUCTION

Diabetic retinopathy is the main reason of visual impairment in the industrial countries and is related to 1%-5% of cases of blindness worldwide[1]. The main reason of vision decrement in diabetic retinopathy is diabetic macular edema (DME) which could be detected during non-proliferative or proliferative stage[2,3]. According to the Wisconsin Epidemiologic Study of Diabetic Retinopathy, the prevalence of DME was 20.1% for type Ⅰ diabetes mellitus and 25.4% for type 2 diabetes mellitus receiving insulin treatment[4].

DME is generally classified into two subtypes. First is the focal edema which consists of localized areas of retinal thickening originating from the leaking micro-aneurysms and is generally associated with hard exudates. Second is the diffuse macular edema which consists of generalized leakage of dilated capillaries and disrupted retinal pigment epithelial barrier[5,6].

DME is associated with hypertension, poor blood glucose regulation, cardiovascular disease, impaired renal function, increased number of microaneurysms and vitreomacular traction[7,8]. Regulation of blood glucose level, systemic hypertension and hyperlipidemia along with following the at-risk patients are the most efficient ways to prevent the vision loss from diabetic retinopathy[2,9].

The gold standard treatment for DME has been macular photocoagulation (MPC) in recent decades[10]. The Early Treatment Diabetic Retinopathy Study (ETDRS) demonstrated that approximately 40% of the patients had achieved ⩾ 6 letters in best corrected visual acuity (BCVA) with focal laser treatment in 3 years[10,11]. Recently, the Diabetic Retinopathy Clinical Research Network (DRCR.net) has demonstrated BCVA improvement of more than 5 letters of vision in 51%, 47% and 62% of eyes treated with MPC after 1, 2 and 3 years of follow-up, respectively[12].

In recent years, alternative or adjunct treatments for DME have been studied, and various pharmacological compounds are under investigation, such as therapies using inhibitors of VEGF[13,14]. The purpose of this assessment is to review the evidence for current anti-VEGF pharmacotherapies in the treatment of DME.

ANTI-VEGF AGENTS FOR DME

The expression of VEGF which stimulates angiogenesis, inflammation and vascular permeability increases due to hypoxia[15]. VEGF molecule breaks down the blood-retinal barrier by its distracting impact on the endothelial zona occludens and induction of fenestrations on the endothelial cells[16,17]. In addition, VEGF causes degeneration in endothelial basement membranes which deteriorate the structure of the retinal microvessels with leakage of blood plasma proteins into the extracellular space[18,19]. The proinflammatory effect of VEGF is related to over-expression of intercellular adhesion molecule-1 which leads leucocyte adhesion to the vascular endothelium, capillary occlusion and endothelial cell apoptosis[20]. VEGF 165 is the leading isoform which is most associated with the increased angiogenesis and vascular permeability[21]. Therefore, VEGF inhibition may be an effective option for management of DME. Several studies have been conducted that have addressed the efficacy and safety of anti-VEGF agents, including ranibizumab (Lucentis, Genentech, Inc., United States), pegaptanib (Macugen, OSI/Eyetech, United States), aflibercept (EYLEA; Regeneron, United States) and bevacizumab (Avastin, Genentech, Inc., United States), in the treatment of DME (Table 1).

CLINICAL TRIALS FOR DME

Pegaptanib sodium (macugen)

Pegaptanib is the first intravitreal VEGF antagonist drug that was approved by the Food and Drug Administration (FDA) for the management of exudative age related macular degeneration (AMD). This molecule is 28-nucleotide chemically synthesized single-stranded nucleic acid (aptamer) that only targets the VEGF 165 isoform[22].

Macugen Diabetic Retinopathy Study Group (a double-masked multicenter controlled phase 2 randomized clinical trial) evaluated the efficacy of pegaptanib in DME[23]. Totally 172 patients with DME who were randomly divided into four arms were enrolled: 0.3, 1, 3 mg intravitreal pegaptanib or sham. Intravitreal pegabtanib injections were administered at weeks 0, 6 and 12. After week 12, additional injections could be performed according to the discrimination of the investigators. In addition focal laser treatment could be chosen as a beginning at week 13. At week 36, better results were achieved in BCVA, central foveal thickness (CFT) and need for additional MPC, in the pegaptanib groups compared to the sham group, in particular the 0.3 mg group. In addition, the better improvements in the pegabtanib groups were determined despite the fact that focal or grid laser was applied 23% more to the sham group between weeks 12 and 36. The proportion of improvements in BCVA was 73% in the 0.3 mg pegabtanib group whereas 51% in the sham group. In detail, the mean increase in BCVA was 4.7 letters and 18% gained 3 or more Snellen lines for the 0.3 mg pegabtanib group. A phase 2/3 randomized, controlled, multicenter trial compared the affectivity and safety of 0.3 mg pegaptanib (administrated for every 6 wk for two years) and sham injections in patients with DME[24]. The total number of subjects included in the first and second year analyses were 260 (133 pegaptanib, 127 sham) and 207 (107 pegaptanib, 100 sham), respectively. The number of patients who gained ⩾ 10 letters in BCVA were 49 (36.8%) and 25 (19.7%) for the pegaptanib and sham groups, respectively, at week 54. At year 1, the BCVA was significantly ($P < 0.05$) improved in the pegaptanib group (gained 5.2 letters) compared to sham (gained 1.2 letters). At year 2, these were 6.1 letters in the pegaptanib group and 1.3 letters in the sham arm ($P < 0.01$).

Table 1 Major trials of anti-vascular endothelial growth factor drugs for diabetic macular edema

Ref.	Drug	Design	*n*	Treatment regimen	Follow-up	Results
Sultan *et al*[24]	Pegaptanib	Phase 2/3, randomized, sham-controlled, multicenter	260 patients	(1) 0.3 mg IVP; or (2) sham injections at baseline and every 6 wk in year 1 and focal/grid laser beginning at wk 18. In year 2, (1) 0.3 mg IVP; or (2) sham up to every 6 wk PRN	2 yr	Improvement of ≥ 10 letters at 54 wk: (1) 36.8%; and (2) 19.7% (P = 0.0047). BCVA letters gained at week 102: (1) 6.1 letters; and (2) 1.3 letters (P < 0.01). No significant difference in CFT decreases at 54 and 102 wk between (1) and (2)
Macugen Diabetic Retinopathy Study Group[23]	Pegaptanib	Phase 2, randomized, double-masked, dose-ranging, controlled	172 patients	(1) 0.3 mg PEG; or (2) sham at baseline, week 6 and week 12; additional injections or focal LPC as needed for an additional 18 wk	36 wk	Mean VA at week 36: (1) 20/50; and (2) 20/63 (P = 0.04). Ten letters gained: (1) 34%; and (2) 10% (P = 0.003). CRT at week 36: (1) -68 μm; and (2) +4 μm (P = 0.02). PEG doses of 0.3, 1, 3 mg all well tolerated
Elman *et al*[28] (DRCR)	Ranibizumab	Randomized, prospective, multicenter	854 eyes of 691 patients	(1) 0.5 mg IVR plus prompt laser; (2) 0.5 mg IVR plus deferred laser (> 24 wk); and (3) 4 mg IVT plus prompt laser; (D) sham injection plus prompt laser	1 yr	Mean VA letter improvement at 1 yr: (1) +9 ± 1, P < 0.001; (2) +9 ± 12, P < 0.001; (3) +4 ± 13, P = 0.31; and (4) +3 ± 13
Mitchell *et al*[33] (RESTORE)	Ranibizumab	Randomized, prospective, multicenter	345 patients	(1) 0.5 mg IVR monthly × 3 then PRN + sham laser; (2) 0.5 mg IVR monthly × 3 then PRN + laser; and (3) sham injections + laser	12 mo	VA better for (1) and (2) from months 1 to 12 compared with (3); 12-mo VA: (1) +6.1 letters; (2) +5.9 letters; and (3) +0.8 letters (P < 0.0001 for both); BCVA 20/40 or better: (1) 53%; (2) 44.9%; and (3) 23.6%. No significant differences between (1) and (2) at 12 mo
RISE Trial[31]	Ranibizumab	Phase 3, randomized, sham-controlled, multicenter	377 patients	(1) 0.3 mg IVR; (2) 0.5 mg IVR; and (3) sham injection. All given monthly injections × 24 mo and with rescue laser available at 3 mo	2 yr	Improvement of ≥ 15 letters at 2 yr: (1) 44.8% (56/125); (2) 39.2% (49/125); and (3) 18.1% (23/127). Statistically significant for both (1) and (2) compared with (3) at P < 0.001 and P < 0.002, respectively
RIDE Trial[31]	Ranibizumab	Phase 3, randomized, sham-controlled, multicenter	382 patients	(1) 0.3 mg IVR; (2) 0.5 mg IVR; and (3) sham injection. All given monthly injections × 24 mo and with rescue laser available at 3 mo	2 yr	Improvement of ≥ 15 letters at 2 yr: (1) 33.6% (42/125); (2) 45.7% (58/127); and (3) 12.3% (16/130). Statistically significant for both (1) and (2) compared with (3) at P < 0.001
Massin *et al*[27] (RESOLVE)	Ranibizumab	Phase 2, randomized, sham controlled, multicenter	151 patients	(1) 0.3 mg or 0.5 mg IVR monthly × 3 mo then as needed (dose doubling allowed after 1 mo); or (2) sham injection monthly × 3 mo then as needed (as-needed rescue LPC in)	1 yr	Month 12 mean ± SD BCVA change: (1) 10.3 ± 9.1 letters; and (2) -1.4 ± 14.2 letters; P < 0.001. Gain ≥ 10 letters: (1) 60.8%; and (2) 18.4% (P < 0.001). Mean change in CFT: (1) -194.2 μm; and (2) -48.4 μm (P < 0.001)
DRCR[41]	Bevacizumab	Randomized, prospective	121 patients	(1) Focal LPC; (2) IVB 1.25 mg at baseline and 6 wk; (3) 2.5 mg IVB at baseline and 6 wk; (4) 1.25 IVB at baseline and sham at 6 wk; or (5) 1.25 IVB at baseline and 6 wk with focal LPC	24 wk	Baseline CFT: 411 μm; at 3 wk, CFT reduction greater in (2) and (3) than in (1); CFT reduced > 11% at 3 wk in 43% of IVB-treated eyes and 28% of LPC treated eyes, and at 6 wk in 37% of IVB treated eyes and 50% of LPC-treated eyes. Mean 12-wk VA improvement in (2) and (3) of 1 line better than (1). No significant short-term benefit combining IVB and laser
Michaelides *et al*[42], 2012 (BOLT)	Bevacizumab	Randomized, prospective	80 patients	(1) Focal/grid laser; or (2) IVB 1.25 mg at baseline, 6 and 12 wk, then as needed	24 mo	Mean gains in BCVA at 24 mo: (1) +2.5 letters; and (2) +9 letters (P = 0.005). Mean change in CFT at 24 mo; (1) -118 μm; and (2) -146 μm
Do DV *et al*[38], 2012 (DA VINCI)	Aflibercept	Phase 2, randomized, multicenter	221 patients	VEGF Trap-Eye (1) 0.5 mg every 4 wk (0.5q4); (2) 2 mg every 4 wk (2q4); (3) 2 mg every 8 wk after 3 initial monthly doses (2q8); (4) 2 mg dosing as needed after 3 initial monthly doses (2PRN); or (5) macular laser photocoagulation.	2 yr	Mean improvements in BCVA in the VEGF Trap-Eye groups at week 52 were 11.0, 13.1, 9.7, and 12.0 letters for 0.5q4, 2q4, 2q8, and 2PRN regimens, respectively, *vs* -1.3 letters for the laser group (P ≤ 0.001 *vs* laser)

BCVA: Best-corrected visual acuity; CFT: Central foveal thickness; DRCR: Diabetic Retinopathy Clinical Research Network; IVB: Intravitreal bevacizumab; PRN: Pro re nata; IVP: Intravitreal pegaptanib; IVR: Intravitreal ranibizumab; IVT: Intravitreal triamcinolone; LPC: Laser photocoagulation; VEGF: Vascular endothelial growth factor.

Ranibizumab (lucentis)

Ranibizumab is a humanized antibody fragment which shows affinity to all VEGF-A isoforms. In 2006, Nguyen *et al*[22] showed the crucial effect of VEGF in DME pathogenesis for the first time and suggested that application of VEGF antagonists such as ranibizumab may reduce retinal edema. Major clinical trials compared the affectivity and safety of ranibizumab with sham or with laser photocoagulation and intravitreal triamcinolone acetonide (IVTA).

The READ-2 study demonstrated that intravitreal ranibizumab achieved better visual results compared to

photocoagulation[25]. Subjects were randomly divided into three groups: 0.5 mg ranibizumab (group 1), focal or grid laser photocoagulation (group 2), or laser plus ranibizumab (group 3). The mean improvement in BCVA was 7.24, 0.43, and 3.8 letters after the primary end point at month 6. At month 24 these were 7.7, 5.1, and 6.8 letters, respectively. The CFT values at month 24 were 340 μm, 286 μm, and 258 μm, respectively. In the ranibizumab group, the mean BCVA (ΔBCVA letters = 3.1, P = 0.009) and CFT (ΔCFT = 70 μm, P = 0.006) were significantly improved at month 36 compared to month 24. However, these were not statistically significant in the laser (-1.6 letters and -36 μm, respectively) and the ranibizumab + laser groups (+2.0 letters and -24 μm). This study showed that long-term results of ranibizumab therapy for DME are favorable, however, injections should be performed frequently in many patients to control edema and maintain the vision[26].

The safety and efficacy of ranibizumab in diabetic macular edema with center involvement study was a multi-center, randomized trial including 151 patients who were administrated either sham, ranibizumab 0.3 mg, or ranibizumab 0.5 mg injections monthly for 3 mo and followed by PRN (Pro Re Nata) treatment[27]. Ranibizumab was increased to 0.6 mg and 1 mg, respectively, if the CFT persisted > 300 μm at the first month or if the CFT was > 225 μm with a decrease in CFT < 50 μm compared to the preceding measurement at any visit following the baseline injection. The injections were interrupted at any monthly visit following the third injection if the CFT was < 225 μm and the BCVA was > 79 letters. The injections were restarted if the CFT increased by > 50 μm or the BCVA worsened ⩾ 5 letters and was < 74 letters. At 12 mo, the improvement in BCVA was 10.2 letters in the ranibizumab group whereas decreased 1 letter in the sham group. Regarding the change in CFT, it was decreased 200 μm in the ranibizumab group and 40 μm in the sham group. The crucial point of this study is to evaluate the outcome of ranibizumab retreatment strategy that could be applicable in clinical practice.

The DRCR.net is a multicenter, randomized clinical trial evaluating whether ranibizumab combined with prompt (within 10 d) or deferred (no sooner than 6 mo) laser, and IVTA combined with prompt laser, might improve BCVA compared to focal/grid photocoagulation alone in central involved DME. At the first year, the mean BCVA significantly improved both in the ranibizumab + prompt laser (+9 ± 11 letters, P < 0.001) and the ranibizumab + deferred laser (+9 ± 12 letters, P < 0.001) groups, however, it was not in the triamcinolone + prompt laser group (+4 ± 13 letters, P = 0.31) compared to the sham + prompt laser group (+3 ± 13 letters). The mean decrease in the CFT was similar between the triamcinolone + prompt laser group and both ranibizumab groups. In addition, these were greater compared to the sham + prompt laser group. Regarding the 3-year results, ranibizumab + prompt laser therapy did not show better BCVA outcomes, and possibly worse, compared to the ranibizumab + deferred laser. They suggested that these BCVA differences may be associated with fewer cumulative ranibizumab injections in the prompt laser treatment group during the follow-up period[28,29]. The 5-year results have recently been reported[30]. The mean BCVA improvement was 7.2 letters in ranibizumab + prompt laser group and 9.8 letters in the ranibizumab + deferred laser group (mean difference was -2.6 letters, P = 0.09). No additional laser treatment was performed in 56% of patients from the deferred laser group during the 5-year follow-up period. The median number of injections in the prompt and deferral groups was 13 and 17, respectively. The percentage of patients receiving no injections in the prompt and deferral groups were 54% and 45% during 4 years of follow-up, respectively, and 62% and 52% during 5 years of follow-up, respectively. The 5-year results demonstrated that BCVA was not significantly different between the ranibizumab + prompt laser and ranibizumab + deferred laser treatment groups. Despite the fact that half of the eyes from the deferred laser treatment group did not receive additional laser treatment during 5 years, more injections were administrated in such eyes to achieve these results. Finally the BCVA improvement was sustained in most eyes from year 1 to 5 with a small number injection after the year 3 in both ranibizumab groups.

The RISE and RIDE are parallel, phase 3, multicenter, sham controlled, randomized studies comparing sham injections with 0.3 or 0.5 mg ranibizumab injections on a monthly basis for 24 mo[31]. Macular laser was available per-protocol–specified criteria. The RISE study showed that the percentage of patients gaining ⩾ 15 letters was 18.1% in sham, 44.8% in 0.3 mg (P < 0.001) and 39.2% in 0.5 mg ranibizumab (P < 0.001) groups. In RIDE, 12.3% of sham patients, 33.6% of 0.3 mg patients (P < 0.001) and 45.7% of 0.5 mg ranibizumab patients (P < 0.0001) gained ⩾ 15 letters. RISE and RIDE studies demonstrated that monthly ranibizumab achieved better improvements in visual acuity than PRN. The FDA approved ranibizumab for the DME treatment based on the satisfactory outcomes of RISE and RIDE. At 36 mo, the percentage of patients gaining ⩾ 15 letters was 22.0% in sham, 51.2% in 0.3 mg (P < 0.001) and 41.6% in 0.5 mg ranibizumab (P < 0.001) groups in RISE, and 19.2%, 36.8% (P < 0.001) and 40.2% (P < 0.001), respectively, in RIDE. These data revealed that the BCVA improvement at month 24 was sustained through month 36[32].

The RESTORE study compared the mean BCVA change in the ranibizumab 0.5 mg monotherapy or combined laser therapy with the laser alone therapy over 12 mo in 345 DME patients[33]. Both ranibizumab groups received three monthly injections followed by PNR injections through the primary end point (month 12). The mean BCVA improvement was 6.1 letters in the ranibizumab monotherapy group, 5.9 letters in the combination group and 0.8 letters in the laser monotherapy group. The percentage of patients who

gained ⩾ 15 letters at month 12 was 26, 27, and 9 for all groups, respectively. At 2 years, the mean BCVA gain observed at month 12 was maintained in the ranibizumab and combined laser groups (7.9 and 6.7 letters, respectively). In the laser alone group, the mean BCVA was improved from month 12 to 24 (5.4 letters) with an average of 4.1 ranibizumab injections[34]. The 3-year results have also been published[35]. The mean BCVA improvement was 8.0 letters in the ranibizumab monotherapy group, 6.7 letters in the combination group with the mean injection numbers of 6.8 and 6.0, respectively. In the laser only group, the mean BCVA improvement was 6.0 letters with a mean of 6.5 ranibizumab injections from month 12 to 36. They suggested that ranibizumab achieves improving and maintaining BCVA with a progressively decreasing number of injections over 3 years

Aflibercept (EYLEA)

Different from ranibizumab and bevacizumab, aflibercept combines the domains of VEGF receptor (VEGFR-1 and VEGFR-2 receptors) to the FC segment of human immunoglobulin G1. It has the highest affinity to all VEGF-A isoforms among anti-VEGF agents. In addition it binds the other VEGF molecules such as placental growth factors 1 and 2 which have been reported to cause an increased vascular permeability[36]. Its efficacy and safety have been evaluated in patients with DME, AMD and retinal vein occlusions. The European Union has recently approved aflibercept for treatments of exudative AMD and retinal vein occlusion and FDA approved for DME treatment.

The DA VINCI is a multicenter, randomized clinical trial comparing the efficacy of aflibercept with laser photocoagulation in DME patients[37,38]. In this study, patients were randomly divided into five aflibercept application groups: 0.5 mg monthly, 2 mg monthly, 2 mg every 8 wk, 2 mg if necessary following 3 initial monthly injections or macular laser treatment. At 24 wk, the increase in BCVA was from 8.5 to 11.4 letters in aflibercept groups and 2.5 letters in the laser group. The BCVA improvement at 52 wk ranged from 9.7 to 12 letters and 1.3 letters, respectively. Regarding the decrease in CFT, it ranged from -165.4 to 227.4 μm in the aflibercept groups and 227.4 to 58.4 μm in the laser groups.

VISTA (DME) and VIVID (DME) were two double-masked, randomized, phase 3 trials comparing the efficacy of 2 mg aflibercept every 4 wk, 2 mg every 8 wk following the 5 incipient monthly doses, with macular laser photocoagulation[39]. At the first year of VISTA, the mean BCVA improvement was 12.5, 10.7 and 0.2 letters, respectively (P < 0.001). These were 10.5, 10.7 and 1.2 letters, respectively (P < 0.001) in the first year of VIVID. The percentages of patients gaining ⩾ 15 letters were 41.6%, 31.1% and 7.8%, respectively (P < 0.001), in VISTA, and 32.4%, 33.3% and 9.1%, respectively (P < 0.001), in VIVID. Regarding the mean CFT decrease, these were 185.9, 183.1 and 73.3 μm, respectively (P < 0.001), in VISTA, and 195.0, 192.4 and 66.2 μm, respectively (P < 0.001), in VIVID. In conclusion, aflibercept groups achieved better functional and anatomic outcomes at the first year compared to the laser group. However, these were similar between the 4 wk and 8 wk injection groups. After two years of VIVID, the mean BCVA improvement for 2 mg aflibercept every 4 wk and 2 mg every 8 wk was 11.4 and 9.4 letters (P < 0.001), respectively, however, it was 0.7 letters for the laser photocoagulation group. Additionally, the percentage of patients gaining ⩾ 15 letters was 38.2% and 31.1% in the 2 mg aflibercept every 4 wk and 2 mg every 8 wk groups, respectively (P < 0.001) compared to the laser photocoagulation group with a percentage of 12.1. These results demonstrated that the improvement in BCVA resumes after two years.

Protocol T, phase 3 study sponsored by the DRCR will compare the safety and efficacy of intravitreal aflibercept (2.0 mg), bevacizumab (1.25 mg) and ranibizumab (0.5 mg) for DME in 660 patients recruited from different clinical centers in the United States. According to the protocol-specified algorithm, the drugs were injected every 4 wk. The primary outcome in this study is to evaluate the changes in BCVA at month 12. At last visit, the mean BCVA improvement score (range, 0 to 100, and a score of 85 is approximately 20/20) was 13.3 with aflibercept, 9.7 with bevacizumab, and 11.2 with ranibizumab. The BCVA improvement was better in aflibercept group (P < 0.001 for bevacizumab and 0.03 for ranibizumab); however, these were not clinically significant because these differences were due to the eyes with worse baseline BCVA (P < 0.001 for interaction). There were no differences in BCVA among the study groups if the baseline visual loss is mild, however, better improvement was achieved by aflibercept at worse initial BCVA[40].

Bevacizumab (avastin)

Bevacizumab is a full-size, humanized, recombinant monoclonal immunoglobulin G which combines all VEGF A isoforms. It is approved by the FDA for colorectal cancer treatment; however, its usage for ocular diseases is off-label. It is widely used for DME treatment due to its favorable cost and availability[6].

DRCR.net is the first study to suggest that bevacizumab warrants phase 3 evaluation for DME treatment[41]. This randomized study evaluated 121 eyes with DME over 12-wk follow-up (safety data are reported for 24 wk). Five treatment groups were studied: (1) focal photocoagulation; (2) 1.25 mg of bevacizumab administrated at 0 and 6 wk; (3) 2.5 mg of bevacizumab administrated at 0 and 6 wk; (4) 1.25 mg of bevacizumab at baseline plus sham injection at 6 wk; and (5) 1.25 mg of bevacizumab at 0 and 6 wk plus focal photocoagulation at 3 wk. Sixty-nine percent of the study eyes had previous DME treatment. BCVA

was significantly improved in the groups receiving two bevacizumab injections compared to the laser group, and this was continued through the 12-wk follow-up period. The increase in BCVA was 7 letters in the 1.25 mg group and 8 letters in the 2.5 mg group at week 9 (following the second injection). Similar to BCVA, these injection groups showed a greater improvement in CFT compared to others with a similar trend in CFT during follow-up. The CFT results did not show any significant difference between the 1.25 and 2.5 mg groups. The results did not show any difference between the single injection group and the photocoagulation group. The laser and bevacizumab combination group showed similar results with the laser-only group. The BCVA results suggested a worsening trend in these two groups different from the two bevacizumab injections groups. In summary, DRCR.net trial revealed that bevacizumab is a favorable agent for treatment of DME in primary cases and also in previously treated DME eyes. This trial identified two trends: (1) Greater improvement is achieved in the primarily treated eyes (P = 0.04) than the refractory eyes; and (2) The initial subretinal fluid may be associated with a greater improvement in BCVA (P = 0.06).

BOLT study is a prospective study comparing bevacizumab treatment with laser in eyes with persistent DME[42]. In this study 80 eyes were randomly assigned into two groups: (1) bevacizumab group (injections applied every 6 wk, with a minimum of 3 and a maximum of 9 injections); and (2) photocoagulation group (performed at 4 mo and a minimum of 1 and a maximum of 4 sessions). After 1 year, the BCVA and CFT results showed greater improvements in the bevacizumab group than in the laser group. After 2 years, the mean BCVA improvement was 9 letters in the bevacizumab and 2.5 letters in laser groups, and 45% of bevacizumab-treated patients had gained 10 or more letters, which was achieved in 7% of the laser group. In addition CFT was significantly decreased in both groups at 2-year follow-up. This study identified two trends: (1) The patients with better baseline BCVA needed fewer injections; and (2) The eyes with subretinal fluid required more injections compared to eyes with diffuse and cystoid edema.

Ahmadieh *et al*[43] performed a randomized study including 115 eyes with DME. Patients were assigned into three groups: bevacizumab-only group (three 1.25 mg bevacizumab injections every 6 wk), IVTA/bevacizumab combination group (additional injection of 2 mg of triamcinolone at the baseline visit only), and placebo group. The first two groups achieved higher improvement in BCVA compared to placebo only with the exception of the bevacizumab monotherapy group at the first 6 wk. Regarding the difference between the first two groups, no significant difference was found for BCVA and CFT. Following the final injection, the effect of bevacizumab continued for 12 wk without any obvious trend of thorough worsening in BCVA and CFT over that period.

Faghihi *et al*[44] compared bevacizumab monotherapy with combined bevacizumab/IVTA and laser in a pure group of patients with no treatment history for DME. Patients received intravitreal injections of 1.25 mg bevacizumab and 2 mg triamcinolone at the initial visit only. CFT was significantly decreased in all groups at both 6 and 16 wk. The bevacizumab monotherapy group had better improvement in BCVA and CFT compared to the laser group at 6 wk but not at 16 wk. However, the combination group achieved better BCVA and CFT at both 6 and 16 wk than the laser group.

Soheilian *et al*[45] compared the efficacy of bevacizumab alone and in combination with IVTA and laser therapy in treatment of DME in a randomized study with 2-year follow-up. Totally 150 eyes were assigned into three groups: 1.25 mg bevacizumab, bevacizumab/IVTA, and bevacizumab/IVTA/laser. The bevacizumab group yielded a significant increase in BCVA at month 6, which was decreased after month 24. In addition the mean BCVA increase was greater in the bevacizumab alone group compared to other study groups. The combined IVTA/bevacizumab group also achieved higher BCVA results than the laser group. Regarding the reduction in CFT, no significant differences were found between groups; however, this may probably be related to study protocol such as the 3-mo retreatment intervals, when indicated, or the missing data in 24.6% of the cases at the final follow-up.

Pan-American Collaborative Retina Study Group performed a retrospective study including DME patients treated with 1.25 mg or 2.5 mg bevacizumab injections[46,47]. At 2-year follow-up, the rate of patients who gained 2 or more ETDR lines was 51.8% whereas 44.6% eyes remained stable, and 3.6% eyes decreased 2 or more ETDRS lines of BCVA. At the last visit, the OCT findings demonstrated that CFT decreased from 446.4 ± 154.4 μm to 279.7 ± 80 μm. The comparison between 1.25 mg and 2.5 mg bevacizumab groups did not reveal any significance in BCVA and CFT.

Different from the other published studies, Haritoglou *et al*[48] included bevacizumab treated DME patients unresponsive to previous treatment, and with diffuse chronic edema. The intravitreal 1.25 mg bevacizumab injections were administrated at baseline, and were repeated based on the BCVA or CFT responses. The mean CFT significantly improved from 463 to 374 μm at 6 mo (P < 0.001).

SAFETY

Pegaptanib has been approved by FDA for the management of exudative AMD. Two clinical studies were performed to study the efficacy and safety of pegaptanib in patients with DME. Cunningham *et al*[23] reported a case of endophthalmitis that occurred in 1 of 652 injections [0.15%/injection; *i.e.*, 1/130 (0.8%) pegaptanib subjects]. In addition, pegaptanib did not show any association with severe BCVA impairment. In the phase 2/3 study[24], the pegaptanib and sham groups were comparable regarding the frequency of

drug interruptions, drug adverse events, treatment-related adverse events and serious adverse events. No case of endophthalmitis or retinal detachment was reported in either treatment group. For serious events cerebrovascular accidents (CVA) were rare, occurring in 2 (1.4%) and in 1 (0.7%) subjects in the pegaptanib and sham arms, respectively. Coronary artery disease and angina pectoris each occurred in 2 (1.4%) pegaptanib treated and 1 (0.7%) sham treated subjects, hypertension was noted for 1 subject in each group (0.07% for both), and unstable angina was experienced by 2 pegaptanib treated and no sham-treated subjects.

Recently ranibizumab has been approved by FDA for treatment of DME. Each of the above mentioned trials for ranibizumab also reported safety data. In these trials, the most common ocular adverse effect is endophthalmitis. In the RISE and RIDE studies there were four total cases of endophthalmitis out of 500 patients in the two-year follow-up of the study (0.8%; 1 in RISE with 0.3 mg ranibizumab, 3 in RIDE, 1 from 0.3 mg group and 2 from 0.5 mg group)[31]. The three-year follow-up of the DRCR study reported a total of 3 cases of endophthalmitis out of 375 (also 0.8%) patients receiving ranibizumab injections, in either the prompt or deferred laser group[29]. The RESTORE study had no cases of endophthalmitis[33]. RESOLVE had 2 cases of endophthalmitis out of 102 injection patients (2%) over the year of the study[27].

The major systemic safety concern with anti-VEGF treatment is thromboembolic events. In the one-year RESTORE study there were 6 arterial thromboembolic events (5.2%) in the ranibizumab (0.5 mg) group, whereas only one such event occurred in the laser group and the laser plus ranibizumab group[33]. The group sizes were similar, and the analysis did not support a statistical difference between ranibizumab treated groups and the laser only group. The one-year RESOLVE study also reported a low incidence of arterial thromboembolic events with no significant difference among treatment groups (3 of 102 in ranibizumab groups, 2 of 49 in sham group)[27]. The three-year follow-up of the DRCR study also reported no significant difference in thromboembolic events in ranibizumab or sham treated groups[29]. In the RISE and RIDE studies, thromboembolic events and deaths were similar between sham and treatment groups[31]. These studies did report that the number of deaths and CVAs were numerically higher in the ranibizumab groups compared to sham groups, with the highest incidences of CVA and death being in the ranibizumab 0.5 mg group. The number of CVAs in the RISE and RIDE studies combined were 4 out of 250 (1.6%), 3 out of 250 (1.2%), and 8 out of 250 (3.2%), in the sham, 0.3 mg, and 0.5 mg groups, respectively. The number of deaths in the combined studies was 3 out of 250 (1.2%), 7 out of 250 (2.8%), and 11 out of 250 (4.4%) in the sham, 0.3 mg, and 0.5 mg groups, respectively.

The largest study evaluating the safety of bevacizumab reported the data from 1173 patients administrated intravitreal bevacizumab and followed for 12 mo[49]. In this retrospective study these following adverse effects were detected: elevated blood pressure in 7 patients, 6 strokes, 5 myocardial infarctions, 5 deaths, bacterial endophthalmitis in 7 patients, tractional retinal detachment in 7 patients, and uveitis in 4 patients. These reported adverse effects were similar to those detected for the other anti-VEGF substances.

The DA VINCI study reported the safety data for aflibercept therapy for DME at one-year follow-up[38]. Similar systemic side effect profile was reported including hypertension (9.7%), cerebral vascular accidents (1.1%), and myocardial infarction (1.1%). The most of ocular side effects were related to intravitreal injection rather than the drug. Serious adverse effects included endophthalmitis (1.1%), uveitis (0.6%), corneal abrasion (0.6%) and retinal tear (0.6%).

Briefly the majority of safety data for anti-VEGF agents come from studies including patients with neovascular AMD; however, the patients with DME tend to be younger, with a high incidence of heart and kidney diseases in addition to the different ocular status. Because the increased rates of neovascularization and fibrous tissue that may lead to contraction and cause additional ocular complications, further safety studies for DME patients are to be necessary.

COST EFFECTIVENESS

To our knowledge, only two cost-effectiveness analyses have evaluated anti-VEGF treatments for DME. Dewan *et al*[50] compared the cost-effectiveness of ranibizumab with that of intravitreal corticosteroids using the data from the DRCRnet study trial and found that ranibizumab met acceptable cost-effectiveness standards relative to intravitreal corticosteroids for phakic patients (those without previous cataract surgery), and intravitreal corticosteroids were the most cost-effective treatment option for pseudophakic patients (those who had undergone cataract surgery). Bevacizumab was not considered in any of their analyses.

Recently Stein *et al*[51] compared the cost-effectiveness of bevacizumab and ranibizumab. They found that intravitreal bevacizumab confers a better value than ranibizumab. They suggest that insurers and health policymakers should consider endorsing the use of intravitreal bevacizumab over other treatment options as first-line therapy for DME, as this may curtail some of the rapidly rising costs of managing patients with this condition.

CONCLUSION

Review of the literature available to date suggests that intravitreal anti-VEGF pharmacotherapy is reasonably safe and effective for the treatment of DME. However, it may be associated with serious complications in spite of the satisfactory improvement in BCVA and macular edema reduction.

Future studies should focus on longer-term safety

and efficacy of anti-VEGF treatment for DME and should evaluate the comparative efficacy of different pharmacologic agents. Future research should also investigate new molecular targets to prevent or delay the progression of DME and novel strategies for sustained intraocular delivery of anti-VEGF agents to reduce the burden, cost, and risks of injections.

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