

Available online at www.sciencedirect.com**ScienceDirect**

Journal of the Chinese Medical Association 78 (2015) 323–330

www.jcma-online.com

Review Article

Corneal neovascularization and contemporary antiangiogenic therapeutics

Chih-Chien Hsu^{a,b}, Hua-Ming Chang^c, Tai-Chi Lin^{a,b}, Kuo-Hsuan Hung^{b,d}, Ke-Hung Chien^{e,f},
Szu-Yu Chen^c, San-Ni Chen^{g,h}, Yan-Ting Chen^{b,g,i,*}

^a Department of Ophthalmology, Taipei Veterans General Hospital, Taipei, Taiwan, ROC

^b Institute of Clinical Medicine, National Yang-Ming University, Taipei, Taiwan, ROC

^c Department of Optics and Photonics, National Central University, Chungli, Taiwan, ROC

^d Department of Ophthalmology, National Yang-Ming University Hospital, Yilan, Taiwan, ROC

^e Department of Ophthalmology, Tri-Service General Hospital, Taipei, Taiwan, ROC

^f Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan, ROC

^g Department of Ophthalmology, Changhua Christian Hospital, Changhua, Taiwan, ROC

^h School of Medicine, Chung-Shan Medical University, Taichung, Taiwan, ROC

ⁱ Department of Optometry, Central Taiwan University of Science and Technology, Taichung, Taiwan, ROC

Received May 5, 2014; accepted October 21, 2014

Abstract

Corneal neovascularization (NV), the excessive ingrowth of blood vessels from conjunctiva into the cornea, is a common sequela of disease insult that can lead to visual impairment. Clinically, topical steroid, argon laser photocoagulation, and subconjunctival injection of bevacizumab have been used to treat corneal NV. Sometimes, the therapies are ineffective, especially when the vessels are large. Large vessels are difficult to occlude and easily recanalized. Scientists and physicians are now dedicated to overcoming this problem. In this article, we briefly introduce the pathogenesis of corneal NV, and then highlight the existing animal models used in corneal NV research—the alkali-induced model and the suture-induced model. Most of all, we review the potential therapeutic targets (i.e., vascular endothelial growth factor and platelet-derived growth factor) and their corresponding inhibitors, as well as the immunosuppressants that have been discovered in recent years by corneal NV studies.

Copyright © 2015 Elsevier Taiwan LLC and the Chinese Medical Association. All rights reserved.

Keywords: angiogenesis; antiangiogenesis; corneal neovascularization; immunosuppressant; vascular endothelial growth factor

1. Introduction

The cornea is the outermost part of the eye, constituting the most important refractive structure of the visual system. Avascularity and transparency of the cornea are vital to normal vision. From the outermost to the innermost layer, the cornea

can be divided into five parts: the epithelium, Bowman's layer, stroma, Descemet's membrane, and endothelium. The epithelium is composed of five to seven layers of cells that are continuous with the conjunctival epithelium. The epithelium is responsible for maintaining the smoothness and integrity of the anterior surface of the cornea. The stromal part is the thickest part of the cornea. Its transparency depends on strictly arranged collagen fibers and endothelial pumping function. Endothelial cells are responsible for water and solute transport between the corneal stroma and the aqueous humor, but are unable to regenerate in humans.

In most tissues, blood and lymphatic vessels are needed to supply oxygen and nutrients, drain extracellular fluid, and

Conflicts of interest: The authors declare that there are no conflicts of interest related to the subject matter or materials discussed in this article.

* Corresponding author. Dr. Yan-Ting Chen, Department of Ophthalmology, Changhua Christian Hospital, 135, Nanhsiao Street, Changhua 500, Taiwan, ROC.

E-mail address: ciloxan@gmail.com (Y.-T. Chen).

<http://dx.doi.org/10.1016/j.jcma.2014.10.002>

1726-4901/Copyright © 2015 Elsevier Taiwan LLC and the Chinese Medical Association. All rights reserved.

protect them against pathogens. However, the cornea is a unique tissue without vascularization. The glucose diffusing from the aqueous humor and oxygen diffusing through the tear film meet the metabolite demand of the cornea, so it can remain avascular in a healthy eye. In some diseased conditions, pathologic corneal hemangiogenesis or lymphangiogenesis occurs, which decreases corneal transparency. Situations leading to corneal angiogenesis are mostly associated with hypoxia, infection, inflammation, and poor limbal barrier function.^{1,2} Accordingly, patients with transplanted cornea, infectious keratitis, ocular chemical or traumatic injury, autoimmune diseases, and chronic contact lens wear are at risk of developing corneal neovascularization (NV). We summarize the etiologies of corneal NV in Table 1.^{1–3}

Angiogenesis, the formation of new vessels from preexisting vascular structures, is different from physiological vasculogenesis. Vasculogenesis means the formation of new blood vessels from bone marrow-derived angioblasts, which mainly occurs during embryogenesis.⁴ Angiogenesis results from an imbalance between angiogenic and antiangiogenic factors.⁵ These factors promote the migration and proliferation of vascular endothelial cells, finally forming a capillary tube as a part of the wound-healing process.^{6,7}

Corneal NV has three clinical patterns. The first one, vascular pannus, results from ocular surface disease. The second is stromal NV, which results from stromal keratitis or alkaline injury. The third is deep NV overlying Descemet's membrane, which can be observed in herpes or interstitial keratitis.^{1,2,5,8,9} Mixed patterns are often seen clinically. Researchers have been working on different strategies for the anti-(lymph) angiogenic therapies of different patterns.

2. Animal models of corneal NV

In order to survey the pathogenesis of corneal hemangiogenesis, lymphangiogenesis, and tissue responses to therapies, scientists have developed some platforms to simulate these conditions. Many different methods have been used to induce corneal NV in animals. Among these, alkali burn and suture

placement are the two animal models that are widely accepted. In the alkali-induced model, corneal NV can be induced by a paper disc soaked with 1N NaOH placed on the animal ocular surface for 10 seconds. The paper disc is later removed, and the ocular surface is washed with normal saline. In the suture-induced model, corneal NV can be triggered by directly suturing two stitches with 10-0 nylon onto the temporal cornea. In both models, corneal NV will appear and progressively extend its occupying area within 2 weeks. Giacomini and colleagues¹⁰ compared these two models in C57BL/6 mice and FVB mice. They found that hemangiogenesis was similar between these two models in C57BL/6 mice, and in the sutured models between C57BL/6 and FVB. Corneal lymphangiogenesis was more pronounced in the sutured group than in the alkali burn group of C57BL/6 mice. More prominent lymphatic vessels were noted in the FVB strain, compared with both models in C57BL/6 mice. Thus, the suture model may be more appropriate for the survey of corneal lymphangiogenesis. Jia and coworkers¹¹ compared the genome-wide gene expression between suture- and alkali-induced murine models by microarray assay. The results pointed out that the overlapping upregulated genes were associated with chemotaxis and immune response, whereas downregulated genes were associated with oxidation reduction and programmed cell death. In both models, vascular endothelial growth factor (VEGF) was upregulated, while pigment epithelium-derived factor (PEDF) remained stable.

Another frequently used method for the *in vivo* survey of corneal NV is corneal micropocket assay, which needs to create two adjacent micropockets on the animal cornea. VEGF or basic fibroblast growth factor (bFGF) is implanted in one micropocket, while the target antiangiogenic agent is implanted in the other. The degree of corneal NV can be measured using a slit-lamp stereomicroscope.

3. Potential targets of therapy

There are many potential targets for antiangiogenic therapies. With new techniques to enhance or suppress these targets, we can reduce corneal NV and maintain corneal transparency. We depicted several important targets and summarized their related therapies in Table 2.^{19–33,36,38,42–46,48,49,54–57,60–62,66,67,71,73,74,77,78,80–87}

3.1. Vascular endothelial growth factor

VEGF is the most important target for antiangiogenic therapies. The so-called VEGF family includes VEGF-A, VEGF-B, VEGF-C, VEGF-D, and placental growth factor.¹² VEGF-A can induce hemangiogenesis through VEGF receptor (VEGFR)-2, whereas VEGF-C and VEGF-D can stimulate lymphangiogenesis through VEGFR-2 and VEGFR-3, respectively.^{13–16} Macrophages in corneal stroma can also produce VEGF-A, VEGF-C, and VEGF-D after injury or inflammation.¹⁷ Therapies that block VEGF may reduce corneal hemangiogenesis and lymphangiogenesis through regulating the signaling pathway of receptor tyrosine kinases,

Table 1
Causes of corneal neovascularization.

Categories	Cause
Hypoxia	Contact lens wearing
Infectious keratitis	Viral Fungal Bacterial Parasitic
Inflammatory disorder	Mucous membrane pemphigoid Stevens–Johnson syndrome Atopic conjunctivitis Rosacea Lyell's syndrome Corneal graft rejection
Loss of limbal barrier function	Limbal stem cell deficiency Chemical burn, thermal burn, or other injury
Other disorders	Ocular surface neoplasia (papilloma, and conjunctival or corneal intraepithelial neoplasia) Pterygium

Table 2
Corneal antiangiogenesis target therapies.^a

Targets	Methods	Therapeutics
Vascular endothelial growth factor	Anti-VEGF-A antibodies	Bevacizumab ^{19–27} Ranibizumab ^{21,23,25,26,28–32} FD006 ³³
	Soluble or modified VEGF receptors	Recombinant dimeric VEGFR-2-Fc ³⁶ sVEGFR-1 overexpression gene therapy ⁴² sVEGFR-3 overexpression gene therapy ³⁸ VEGFR-1 morpholino ⁴³ Aflibercept/VEGF-Trap _(R1R2) ^{44,45} VEGFR intrareceptor gene therapy (Flt23k, Flt24k) ^{46,48,49}
Pigment epithelium-derived factor	VEGF-A aptamer	Pegaptanib ^{23,26}
	PEDF direct effect	PEDF ⁵⁴ PEDF-derived peptide ⁵⁵ PEDF gene therapy ^{56,57}
Platelet-derived growth factor	PDGF receptor inhibitor	AG 1296 ^{60,61}
	Multitargeted receptor tyrosine kinase inhibitor	Sunitinib ⁶²
Angiostatin	Angiostatin direct effect	Angiostatin pump ⁶⁶
Hypoxia-inducible factors	shRNA for hypoxia-inducible factors	HIF-1 α shRNA gene therapy (HIF-1 α RNAi-A) ⁶⁷
12-Hydroxyeicosatrienoic acid	siRNA for cytochrome P450 mono-oxygenase	CYP4B1 siRNA gene therapy ⁷¹
Vascular adhesion protein	VAP-1/SSAO inhibitor	U–V002 ⁷³
		LJP1207 ⁷⁴
Decorin	Decorin direct effect	Decorin gene therapy ⁷⁷
Vasohibin-1	Vasohibin-1 directly effect	Vasohibin-1 gene therapy ⁷⁸
Cannabinoid receptor CB1	CB1 antagonist	Rimonabant ⁸⁰

CYP = cytochrome P450 mono-oxygenase; HIF-1 α = hypoxia-inducible factor 1 α ; PDGF = platelet-derived growth factor; PEDF = pigment epithelium-derived factor; SSAO = semicarbazide-sensitive amine oxidase; sVEGFR = soluble form of vascular endothelial growth factor receptor; VAP-1 = vascular adhesive protein-1; VEGF = vascular endothelial growth factor; VEGFR = vascular endothelial growth factor receptor.

^a Other potential therapies: steroid (dexamethasone, prednisolone, fluorometholone), rapamycin, cyclosporine A, thalidomide analogue (CC-3052).^{81–87}

VEGFR-2, and VEGFR-3.^{8,18} Many different strategies have been developed to block the signaling pathway of VEGF-A, for example, monoclonal anti-VEGF-A immunoglobulin or fragment antigen-binding (Fab fragment), fusion protein, aptamer, decoy or soluble VEGFR, and anti-VEGFR antagonist. These strategies will be discussed in more detail below.

3.2. Anti-VEGF-A antibodies

Many anti-VEGF-A antibodies are now available in the market and act as popular antiangiogenic therapies. Bevacizumab is a whole anti-VEGF-A immunoglobulin, widely used in many retinal disorders such as choroidal NV, diabetic retinopathy, and retinal vein occlusion by intravitreal injections. Some researchers have also tried to use bevacizumab in corneal NV. When bevacizumab was administered in a high-risk murine corneal transplantation model, it could significantly reduce corneal NV and increase graft survival by the subconjunctival route rather than by the topical route.¹⁹ Similar conclusions were also drawn by randomized clinical trials on high-risk corneal transplantation in humans.²⁰ The effect of bevacizumab in suture- or burn-induced animal models of corneal NV was also confirmed by the decrease in the area of corneal NV after subconjunctival injections^{21–26} or topical administration.²² With persistent topical administration, the therapeutic effect could even last longer than infrequent early-phase subconjunctival injections in a silver/potassium nitrate cauterized model.²¹ However, when the

topical or subconjunctival administration of bevacizumab was postponed 2 weeks after suture injury in an animal model, its effect of reducing corneal NV was not so significant.²⁷

Ranibizumab is a fab fragment of antibody against VEGF-A. When ranibizumab was administered by the subconjunctival route in a high-risk murine corneal transplantation model, it could significantly reduce corneal NV and increase graft survival.²⁸ The effect of ranibizumab in suture- or burn-induced animal models of corneal NV has also been studied, and the results also showed promise for the reduction of corneal NV by subconjunctival injections^{23,25,26,29,30} or topical administration.^{29,31}

Researchers even compared the therapeutic effect between bevacizumab and ranibizumab in animal models and had diverse findings from different settings. Some studies demonstrated no difference between the two agents with subconjunctival injection,²¹ whereas others showed relatively less NV,^{23,25,26} shorter blood vessels,^{23,25} lower corneal opacity score,²⁵ and lower degree of inflammation²⁶ in the groups treated with bevacizumab compared to those treated with ranibizumab. However, a small, prospective, open-labeled, nonrandomized clinical study showed that ranibizumab was superior to bevacizumab in the onset of action and degree of efficacy when administered by the topical route.³² Such different outcomes might be related to the difference between these two antibodies in their molecular sizes and the ability to penetrate the cornea. By contrast, another new anti-VEGF-A antibody, called FD006, with even higher binding

affinity to VEGF than bevacizumab, was developed. In an alkali-induced model, FD006 was a slightly more effective in reducing corneal NV, compared to bevacizumab.³³

3.3. Soluble or modified VEGF receptors

Some researchers have pointed out that the expression of soluble VEGF receptors by alternative splicing or proteolytic shedding is a natural mechanism to inhibit hemangiogenesis/lymphangiogenesis and maintain avascularity of the cornea.^{34–38} Soluble VEGF receptors can block the effect of VEGF ligand by VEGF trapping and preventing their binding to membrane-bound VEGF receptors.

The soluble truncated form of VEGFR-1, called fms-like tyrosine kinase (sFlt-1, sVEGFR-1), has high affinity to VEGF-A and is essential to maintain corneal avascularity during development.³⁴ In a previous study, strongly expressed sVEGFR-1 in epithelium was found in human corneal tissues harvested from residual corneal graft, but it was weakly expressed in the stroma, while neovascularized corneas showed greatly reduced sVEGFR-1 expression.³⁵ Similarly, VEGFR-2 also has its soluble form (sVEGFR-2). The increased expression of sVEGFR-2 was observed in a suture-induced model, inhibiting lymphangiogenesis and enhancing corneal allograft survival by blocking VEGF-C function.³⁶ However, monomeric sVEGFR-2 could not inhibit corneal hemangiogenesis mediated by VEGF-A in a suture-induced model due to its poor affinity to VEGF-A. Conversely, recombinant dimeric VEGFR-2-Fc, which has a better affinity to VEGF-A,^{39–41} could inhibit corneal hemangiogenesis in the same model.³⁶ Recently, a novel truncated soluble isoform of VEGFR3 (sVEGFR-3) was also reported.³⁸ It could bind with VEGF-C and inhibit lymphangiogenesis of the cornea. When the cornea was injured in a suture-induced model, low levels of sVEGFR-3 and higher levels of VEGFR-3 were observed in the sutured site. Similarly, when sVEGFR-3 was knocked down by shRNA, an increased level of free VEGF-C can be observed, followed by increased expression of membrane-bound VEGFR-3 and formation of lymphatic vessels.³⁸ These findings give us some new ideas for inhibiting hemangiogenesis and lymphangiogenesis by overexpression of soluble VEGF receptors via the following two mechanisms: (1) using soluble VEGF receptors directly or (2) using recombinant fusion proteins of the soluble VEGF receptors. Singh et al³⁸ tried to overexpress sVEGFR-3 by intrastromal injection of plasmid pCMV.sVEGFR-3 in a suture-induced model and a penetrating keratoplasty model. Encouragingly, they found that this method could decrease 58% of the lymphatic area and 31% of the blood vessel area in the sutured cornea model, and also increase the survival rate by 40% in the corneal transplant model. In addition, soluble VEGFR-3 was further proved, from their immunoprecipitation results, to suppress hemangiogenesis by blocking VEGF-C-induced VEGFR-2 phosphorylation.

Employing nanotechnology, Iriyama et al⁴² used poly(ethylene glycol) (PEG)-block-polycation, carrying

ethylenediamine units in the side chains {PEG-b-P [Asp(DET)]}, to form polyplex micelles containing sVEGFR-1 (sFlt-1) plasmid. By subconjunctival injection of these nanoparticles, the prolonged gene expression of sFlt-1 significantly reduced corneal NV in a suture-induced corneal model. Another study tested the effect of VEGFR-1 morpholino in a keratoplasty model through subconjunctival injection. The investigators also found that VEGFR-1 morpholino decreased angiogenesis/lymphangiogenesis and increased graft survival.⁴³

A new recombinant fusion protein, named aflibercept [or VEGF-Trap_(R1R2)], has also been developed recently for anti-angiogenic therapy. Aflibercept recombinant fusion protein has two main components; the VEGF-binding portions from the extracellular domains of human VEGF receptors 1 and 2 are further fused to the Fc portion of human immunoglobulin 1 to form the antibody. This antibody has the ability to bind VEGF-A, VEGF-B, and placental growth factor, so as to inhibit the original signaling pathway. Oliveira et al⁴⁴ tested the effect of intraperitoneal injection of VEGF-Trap_(R1R2) in a bFGF-induced mouse model of corneal NV and found that this method could reduce corneal NV significantly. Similarly, Bachmann et al⁴⁵ tested the effect of intraperitoneal injection of VEGF-Trap after penetrating keratoplasty in a mouse model and found reduced corneal revascularization with better graft survival.

There still exists another concept for blocking VEGF by using VEGFR intraceptor. KDEL is a quadriptide retention signal (Lys–Asp–Glu–Leu) that binds to endoplasmic reticulum retention receptors. It can prevent the secretion of proteins that are coupled to KDEL.⁴⁶ When domains 2 and 3 of VEGFR-1 are coupled to KDEL, the recombinant construct can bind VEGF intracellularly and block the function of VEGF. When domain 4 is also coupled to KDEL, the recombinant construct can even be dimerized with VEGFR-2.⁴⁷ Two types of VEGFR intraceptors, Flt23k (coupling domains 2–3 of Flt with KDEL) and Flt24k (coupling domains 2–4 of Flt with KDEL), have been used as therapeutic agents. Singh et al⁴⁸ tested the effect of gene therapy by intrastromal injection of pCMV.Flt23K or pCMV.Flt24K. They found pCMV.Flt23K and pCMV.Flt24K injections could sequester hypoxia-induced VEGF and reduce alkali-induced VEGF elevation, leucocyte infiltration, and corneal NV. They also found that Flt24K could bind VEGFR-2 in their *in vitro* study.⁴⁷ Furthermore, Jani et al⁴⁶ investigated the effect of intrastromal injection of albumin nanoparticles encapsulating pCMV.Flt23K 3 weeks “prior to” corneal injury in an alkali-induced model. They found that Flt23K could be expressed for a much longer time by nanoparticles encapsulating plasmids (5 weeks) than by naked plasmids (8 days). This result validated that albumin nanoparticles could prolong the expression of therapeutic plasmids. The effect of Flt23k in the form of poly L-lactide-co-glycolide nanoparticles was tested by Cho et al.⁴⁹ They performed this study in a murine cornea transplant model and found that subconjunctival injection of Flt23k poly L-lactide-co-glycolide nanoparticle could reduce NV,

lymphangiogenesis, and graft failure rate. In addition, combined Flt23k nanoparticle and steroid also elevated the 2-month graft survival rate significantly.

3.4. Another VEGF-A antagonist

VEGF-A aptamer pegaptanib is a single-strand RNA that can bind with VEGF-165 to block its effect on angiogenesis or vessel permeability. Some studies showed that pegaptanib could also reduce corneal NV in a burn-induced animal model by subconjunctival injection, but the effect was less than that of bevacizumab on the area of corneal NV and degree of inflammation.^{23,26}

3.5. Pigment epithelium-derived factor

PEDF is a glycoprotein with antiangiogenic, anti-tumorigenic, and neurotrophic functions. PEDF can inhibit VEGF, FGF, and interleukin-8 (IL-8/CXCL8)-mediated angiogenesis by reducing endothelial cell migration and inducing the cells' apoptosis at the same time.^{50–52} PEDF was also found to play a critical role in the antiangiogenic effect of amniotic membrane transplantation.⁵³ Some researchers found that topical PEDF or PEDF-derived peptides (P5-2 and P5-3 peptides) could downregulate VEGF expression and inhibit corneal NV in a chemical-induced corneal model.^{54,55} Some other researchers used Synthetic Amphiphile INteraction-18 (SAINT-18) as a vector to deliver plasmid PEDF by subconjunctival injection in a bFGF-induced corneal NV model and inhibited corneal NV successfully.^{56,57}

3.6. Platelet-derived growth factor

Platelet-derived growth factors (PDGFs) are proteins involved in cell growth, division, angiogenesis, and tissue remodeling. Ligands such as PDGF-A and PDGF-B, and receptors such as PDGFR- α , PDGFR- β can be found in corneal tissue and are associated with angiogenesis of the cornea.^{58,59} Some studies found that intraperitoneal injection of PDGF receptor inhibitor (AG 1296) resulted in the loss of capillary pericytes and reduction of vessel density in advanced corneal NV.^{60,61} In addition, another study pointed out that topically administered Sunitinib, a multitargeted receptor tyrosine kinase inhibitor blocking both PDGF and VEGF, could reduce corneal NV more effectively in a suture-induced model than bevacizumab.⁶²

3.7. Angiostatin

Angiostatin is an endogenous angiogenesis inhibitor cleaved from plasminogen. Angiostatin can bind with many surface proteins in vascular endothelial cells and inhibit their migration, as well as tubule formation.^{63,64} Angiostatin is also produced in the cornea. Its depletion through utilization of artificial antiangiostatin antibodies could increase corneal NV in an animal excimer laser keratectomy model.⁶⁵ With the implantation of an angiostatin pump, corneal NV was also reduced in an alkali-induced model.⁶⁶

3.8. Hypoxia-inducible factors

In hypoxia-induced NV, hypoxia-inducible factor 1 α (HIF-1 α) can bind to the promoter of VEGF gene and activate the transcription of VEGF. Therefore, Chen et al⁶⁷ designed a recombinant vector, named HIF-1 α RNAi-A, to express HIF-1 α -specific shRNA. They tested the effect of shRNA by subconjunctival injection in a contact-lens-wearing-with-tarsorrhaphy-induced corneal NV model and successfully inhibited corneal NV by reducing the expression of VEGF, metalloproteinase (MMP)-2/9, and IL-1 β .

3.9. 12-Hydroxyeicosatrienoic acid

Corneal inflammation or hypoxia-induced angiogenesis is related to 12-hydroxyeicosatrienoic acid, an inflammatory and angiogenic eicosanoid. This acid is metabolized by cytochrome P450 mono-oxygenase (CYP), and its elevated expression with prominent activity could be observed in a contact lens-induced hypoxia model.^{68–70} Seta et al⁷¹ showed that siRNA targeting at CYP4B1 could inhibit not only the level of 12-hydroxyeicosatrienoic acid, but also the expression of VEGF, a reduced corneal NV, in a suture-induced model through subconjunctival injection.

3.10. Vascular adhesion protein

Vascular adhesive protein-1/semicarbazide-sensitive amine oxidase (VAP-1/SSAO) is involved in the adhesion of lymphocytes to endothelial cells and is thought to be related to corneal NV.⁷² Murine corneal micropocket assay showed that the VAP-1 inhibitor, U-V002, reduced the IL-1 β -induced M2 macrophage infiltration and lymphangiogenesis/hemangiogenesis in the cornea, but failed in the VEGF-A-induced lymphangiogenesis/hemangiogenesis.⁷³ Another study showed that corneal VAP-1/SSAO activity rose after suture stimulation in a rabbit suture-induced corneal model. VAP-1/SSAO activity could be reduced by eye drops containing VAP-1/SSAO inhibitor LJP1207, but not by bevacizumab alone. However, LJP 1207 treatment alone could not significantly reduce corneal NV.⁷⁴

3.11. Decorin

Decorin is a small leucine-rich proteoglycan that can suppress endothelial migration and angiogenesis. Its deficiency led to impaired angiogenesis in injured murine cornea.⁷⁵ Some evidence showed that membrane type 1-matrix metalloproteinase (MT1-MMP) was upregulated in bFGF-induced corneal micropocket assay, in which decorin was cleaved by MT-1-MMP in a time- and concentration-dependent manner.⁷⁶ Mohan et al⁷⁷ further tested the effect of targeted decorin gene therapy by topical application of AAV5-dcn vector onto the de-epithelial stroma in micropocket corneal NV assay. They found that decorin gene therapy could significantly suppress corneal NV by downregulating VEGF, macrophage chemoattractant protein (MCP1), and angiopoietin, and upregulating PEDF at the same time.

3.12. Vasohibin-1

Vasohibin-1 is an endothelium-derived negative feedback regulator of angiogenesis. Zhou et al.⁷⁸ tried subconjunctival injection of a recombinant adenovirus encoding human vasohibin-1 in an alkali-induced mouse model, revealing downregulation of VEGF-R2-expression combined with significant reduction of corneal NV.

3.13. Cannabinoid receptor CB1

Cannabinoid receptor subtype 1 is a highly expressed receptor in the central nervous system, and can also be found in ciliary epithelium, corneal epithelium, the endothelium of human anterior segment, and retina.⁷⁹ It can be activated by endocannabinoids and is a potential target for anti-inflammation or antiangiogenesis. Pisanti et al.⁸⁰ used selective CB1 antagonist rimonabant in a bFGF-stimulated rabbit corneal angiogenesis assay and reported a remarkably reduced angiogenesis by using rimonabant.

4. Immunosuppressant

4.1. Steroid

Steroid is the most widely used immunosuppressant clinically. It is also frequently used in ocular disorders to reduce ocular inflammation due to infection, immune response, or ocular surgery. Steroid was found to reduce the development of corneal NV by reducing inflammatory cell recruitment and the level of cytokine, but it could not effectively reduce pre-existing mature corneal NV. An alkali-induced corneal model showed that subconjunctival injection of dexamethasone reduced the intensity of inflammation and area of corneal NV.⁸¹ Another study, of a murine suture-induced corneal model, revealed that topical steroids (dexamethasone, prednisolone, and fluorometholone) can reduce both hemangiogenesis and lymphangiogenesis to different degrees.⁸²

Some studies also looked into the relationship between the effect and timing of steroid administration. In a study of IL-1 β -induced corneal micropocket assay, corneal NV could be reduced by topical dexamethasone when it was used within 4 days after IL-1 β implantation.⁸³ Another study using a murine keratoplasty model found that combined pre- and post-treatment of subconjunctival glucocorticoid led to less hemangiogenesis and lymphangiogenesis, compared with the result of using post-treatment only.⁸⁴ By contrast, both pre- and post-treatment of glucocorticoid showed similar effects of reducing hemangiogenesis and lymphangiogenesis in a murine suture corneal model.⁸⁴

4.2. Rapamycin and cyclosporine A

Some studies tested the effect of other immunosuppressants, such as rapamycin and cyclosporine A, in the treatment of corneal NV. Topical rapamycin was found to be effective in reducing angiogenesis and necrosis in a model of HSV-1

stromal keratitis.⁸⁵ Another study showed that using 0.5% rapamycin eye drop of nanometer vector twice per day alone or combined with poly(lactic acid) wafers of cyclosporine A in the anterior chamber could reduce inflammatory cellular infiltrate and the expression of VEGF gene in a rabbit corneal allograft model.⁸⁶

4.3. Thalidomide analogue CC-3052

Thalidomide is an immunomodulatory and anti-inflammatory agent, but it also has teratogenic and neurotoxic effects. Thalidomide can inhibit tumor necrosis factor- α (TNF- α) and FGF-2-mediated angiogenesis and act as a potential inhibitor of angiogenesis. Lee and Chung⁸⁷ used a water-soluble analog of thalidomide, CC-3052, as a new potential medication for corneal NV in a suture-induced model. They found that topical administration of CC-3052 twice-a-day reduced corneal NV.

In conclusion, corneal angiogenesis takes place by upregulation of angiogenic factors and/or downregulation of anti-angiogenic factors. In order to prevent or treat corneal NV, these angiogenesis-related factors can be manipulated by means of monoclonal antibodies, modified receptors, aptamers, and inhibitors of possible angiogenesis pathways. We have summarized these recently discovered therapeutic targets in this review article. However, further studies are still required to confirm the efficacy and safety of these novel therapies in clinical practice.

References

1. Menzel-Severing J. Emerging techniques to treat corneal neovascularisation. *Eye* 2012;**26**:2–12.
2. Tshionyi M, Shay E, Lunde E, Lin A, Han KY, Jain S, et al. Hemangiogenesis and lymphangiogenesis in corneal pathology. *Cornea* 2012;**31**:74–80.
3. Cursiefen C, Colin J, Dana R, Diaz-Llopis M, Faraj LA, Garcia-Delpech S, et al. Consensus statement on indications for anti-angiogenic therapy in the management of corneal diseases associated with neovascularisation: outcome of an expert roundtable. *Br J Ophthalmol* 2012;**96**:3–9.
4. Azar DT. Corneal angiogenic privilege: angiogenic and antiangiogenic factors in corneal avascularity, vasculogenesis, and wound healing (an American Ophthalmological Society thesis). *Trans Am Ophthalmol Soc* 2006;**104**:264–302.
5. Chang JH, Gabison EE, Kato T, Azar DT. Corneal neovascularization. *Curr Opin Ophthalmol* 2001;**12**:242–9.
6. Stromblad S, Cheres DA. Integrins, angiogenesis and vascular cell survival. *Chem Biol* 1996;**3**:881–5.
7. Lee HS, Chung SK. The effect of subconjunctival suramin on corneal neovascularization in rabbits. *Cornea* 2010;**29**:86–92.
8. Chang JH, Garg NK, Lunde E, Han KY, Jain S, Azar DT. Corneal neovascularization: an anti-VEGF therapy review. *Surv Ophthalmol* 2012;**57**:415–29.
9. Gonzalez L, Loza RJ, Han KY, Sunoqrot S, Cunningham C, Purta P, et al. Nanotechnology in corneal neovascularization therapy: a review. *J Ocul Pharmacol Ther* 2013;**29**:124–34.
10. Giacomini C, Ferrari G, Bignami F, Rama P. Alkali burn versus suture-induced corneal neovascularization in C57BL/6 mice: an overview of two common animal models of corneal neovascularization. *Exp Eye Res* 2014;**121**:1–4.

11. Jia C, Zhu W, Ren S, Xi H, Li S, Wang Y. Comparison of genome-wide gene expression in suture- and alkali burn-induced murine corneal neovascularization. *Mol Vis* 2011;**17**:2386–99.
12. Neufeld G, Cohen T, Gengrinovitch S, Poltorak Z. Vascular endothelial growth factor (VEGF) and its receptors. *FASEB J* 1999;**13**:9–22.
13. Goldman J, Rutkowski JM, Shields JD, Pasquier MC, Cui Y, Schmokel HG, et al. Cooperative and redundant roles of VEGFR-2 and VEGFR-3 signaling in adult lymphangiogenesis. *FASEB J* 2007;**21**:1003–12.
14. Achen MG, Jeltsch M, Kukk E, Makinen T, Vitali A, Wilks AF, et al. Vascular endothelial growth factor D (VEGF-D) is a ligand for the tyrosine kinases VEGF receptor 2 (Flk1) and VEGF receptor 3 (Flt4). *Proc Natl Acad Sci U S A* 1998;**95**:548–53.
15. Joukov V, Pajusola K, Kaipainen A, Chilov D, Lahtinen I, Kukk E, et al. A novel vascular endothelial growth factor, VEGF-C, is a ligand for the Flt4 (VEGFR-3) and KDR (VEGFR-2) receptor tyrosine kinases. *EMBO J* 1996;**15**:290–8.
16. Shibuya M. Vascular endothelial growth factor (VEGF) and its receptor (VEGFR) signaling in angiogenesis: a crucial target for anti- and pro-angiogenic therapies. *Genes Cancer* 2011;**2**:1097–105.
17. Cursiefen C, Chen L, Borges LP, Jackson D, Cao J, Radziejewski C, et al. VEGF-A stimulates lymphangiogenesis and hemangiogenesis in inflammatory neovascularization via macrophage recruitment. *J Clin Invest* 2004;**113**:1040–50.
18. Maddula S, Davis DK, Burrow MK, Ambati BK. Horizons in therapy for corneal angiogenesis. *Ophthalmology* 2011;**118**:591–9.
19. Dastjerdi MH, Saban DR, Okanobo A, Nallasamy N, Sadrai Z, Chauhan SK, et al. Effects of topical and subconjunctival bevacizumab in high-risk corneal transplant survival. *Invest Ophthalmol Vis Sci* 2010;**51**:2411–7.
20. Bhatti N, Qidwai U, Hussain M, Kazi A. Efficacy of sub-conjunctival and topical bevacizumab in high-risk corneal transplant survival. *J Pak Med Assoc* 2013;**63**:1256–9.
21. Kim EK, Kong SJ, Chung SK. Comparative study of ranibizumab and bevacizumab on corneal neovascularization in rabbits. *Cornea* 2014;**33**:60–4.
22. Kim J, Kim D, Kim ES, Kim MJ, Tchah H. Topically administered bevacizumab had longer standing anti-angiogenic effect than subconjunctivally injected bevacizumab in rat corneal neovascularization. *Int J Ophthalmol* 2013;**6**:588–91.
23. Akar EE, Oner V, Kucukerdonmez C, Aydin Akova Y. Comparison of subconjunctivally injected bevacizumab, ranibizumab, and pegaptanib for inhibition of corneal neovascularization in a rat model. *Int J Ophthalmol* 2013;**6**:136–40.
24. Ozdemir O, Altintas O, Altintas L, Yildiz DK, Sener E, Caglar Y. Effects of subconjunctivally injected bevacizumab, etanercept, and the combination of both drugs on experimental corneal neovascularization. *Can J Ophthalmol* 2013;**48**:115–20.
25. Dursun A, Arici MK, Dursun F, Ozec AV, Toker MI, Erdogan H, et al. Comparison of the effects of bevacizumab and ranibizumab injection on corneal angiogenesis in an alkali burn induced model. *Int J Ophthalmol* 2012;**5**:448–51.
26. Sener E, Yuksel N, Yildiz DK, Yilmaz B, Ozdemir O, Caglar Y, et al. The impact of subconjunctivally injected EGF and VEGF inhibitors on experimental corneal neovascularization in rat model. *Curr Eye Res* 2011;**36**:1005–13.
27. Ivacic IS, Goktas S, Sakarya Y, Ozcimen M, Sakarya R. Inhibition of corneal neovascularization by subconjunctival and topical bevacizumab and sunitinib in a rabbit model. *Cornea* 2013;**32**:e193.
28. Cho KJ, Choi JS, Choi MY, Joo CK. Effects of subconjunctival ranibizumab in a presensitized rat model of corneal graft. *Exp Eye Res* 2013;**107**:74–9.
29. Turkcu FM, Cinar Y, Turkcu G, Sahin A, Cingu AK, Yuksel H, et al. Topical and subconjunctival ranibizumab (Lucentis) for corneal neovascularization in experimental rat model. *Cutan Ocul Toxicol* 2014;**33**:138–44.
30. Liarakos VS, Papaconstantinou D, Vergados I, Douvali M, Theodossiadi PG. The effect of subconjunctival ranibizumab on corneal and anterior segment neovascularization: study on an animal model. *Eur J Ophthalmol* 2014;**24**:299–308.
31. Bucher F, Parthasarathy A, Bergua A, Onderka J, Regenfuss B, Cursiefen C, et al. Topical ranibizumab inhibits inflammatory corneal hem- and lymphangiogenesis. *Acta Ophthalmol* 2014;**92**:143–8.
32. Stevenson W, Cheng SF, Dastjerdi MH, Ferrari G, Dana R. Corneal neovascularization and the utility of topical VEGF inhibition: ranibizumab (Lucentis) vs bevacizumab (Avastin). *Ocul Surf* 2012;**10**:67–83.
33. Wang Q, Yang J, Tang K, Luo L, Wang L, Tian L, et al. Pharmacological characteristics and efficacy of a novel anti-angiogenic antibody FD006 in corneal neovascularization. *BMC Biotechnol* 2014;**14**:17.
34. Ambati BK, Nozaki M, Singh N, Takeda A, Jani PD, Suthar T, et al. Corneal avascularity is due to soluble VEGF receptor-1. *Nature* 2006;**443**:993–7.
35. Ambati BK, Patterson E, Jani P, Jenkins C, Higgins E, Singh N, et al. Soluble vascular endothelial growth factor receptor-1 contributes to the corneal antiangiogenic barrier. *Br J Ophthalmol* 2007;**91**:505–8.
36. Albuquerque RJ, Hayashi T, Cho WG, Kleinman ME, Dridi S, Takeda A, et al. Alternatively spliced vascular endothelial growth factor receptor-2 is an essential endogenous inhibitor of lymphatic vessel growth. *Nat Med* 2009;**15**:1023–30.
37. Pavlakovic H, Becker J, Albuquerque R, Wilting J, Ambati J. Soluble VEGFR-2: an antilymphangiogenic variant of VEGF receptors. *Ann N Y Acad Sci* 2010;**1207**(Suppl. 1):E7–15.
38. Singh N, Tiem M, Watkins R, Cho YK, Wang Y, Olsen T, et al. Soluble vascular endothelial growth factor receptor 3 is essential for corneal alymphaticity. *Blood* 2013;**121**:4242–9.
39. Roeckl W, Hecht D, Sztajer H, Waltenberger J, Yayon A, Weich HA. Differential binding characteristics and cellular inhibition by soluble VEGF receptors 1 and 2. *Exp Cell Res* 1998;**241**:161–70.
40. Fuh G, Li B, Crowley C, Cunningham B, Wells JA. Requirements for binding and signaling of the kinase domain receptor for vascular endothelial growth factor. *J Biol Chem* 1998;**273**:11197–204.
41. Wiesmann C, Fuh G, Christinger HW, Eigenbrot C, Wells JA, de Vos AM. Crystal structure at 1.7 Å resolution of VEGF in complex with domain 2 of the Flt-1 receptor. *Cell* 1997;**91**:695–704.
42. Iriyama A, Usui T, Yanagi Y, Amano S, Oba M, Miyata K, et al. Gene transfer using micellar nanovectors inhibits corneal neovascularization *in vivo*. *Cornea* 2011;**30**:1423–7.
43. Cho YK, Zhang X, Uehara H, Young JR, Archer B, Ambati B. Vascular endothelial growth factor receptor 1 morpholino increases graft survival in a murine penetrating keratoplasty model. *Invest Ophthalmol Vis Sci* 2012;**53**:8458–71.
44. Oliveira HB, Sakimoto T, Javier JA, Azar DT, Wiegand SJ, Jain S, et al. VEGF Trap(R1R2) suppresses experimental corneal angiogenesis. *Eur J Ophthalmol* 2010;**20**:48–54.
45. Bachmann BO, Luetjen-Drecoll E, Bock F, Wiegand SJ, Hos D, Dana R, et al. Transient postoperative vascular endothelial growth factor (VEGF)-neutralisation improves graft survival in corneas with partly regressed inflammatory neovascularisation. *Br J Ophthalmol* 2009;**93**:1075–80.
46. Jani PD, Singh N, Jenkins C, Raghava S, Mo Y, Amin S, et al. Nanoparticles sustain expression of Flt intraceptors in the cornea and inhibit injury-induced corneal angiogenesis. *Invest Ophthalmol Vis Sci* 2007;**48**:2030–6.
47. Singh N, Jani PD, Suthar T, Amin S, Ambati BK. Flt-1 intraceptor induces the unfolded protein response, apoptotic factors, and regression of murine injury-induced corneal neovascularization. *Invest Ophthalmol Vis Sci* 2006;**47**:4787–93.
48. Singh N, Amin S, Richter E, Rashid S, Scoglietti V, Jani PD, et al. Flt-1 intraceptors inhibit hypoxia-induced VEGF expression *in vitro* and corneal neovascularization *in vivo*. *Invest Ophthalmol Vis Sci* 2005;**46**:1647–52.
49. Cho YK, Uehara H, Young JR, Tyagi P, Kompella UB, Zhang X, et al. Flt23k nanoparticles offer additive benefit in graft survival and anti-angiogenic effects when combined with triamcinolone. *Invest Ophthalmol Vis Sci* 2012;**53**:2328–36.
50. Dawson DW, Volpert OV, Gillis P, Crawford SE, Xu H, Benedict W, et al. Pigment epithelium-derived factor: a potent inhibitor of angiogenesis. *Science* 1999;**285**:245–8.

51. Duh EJ, Yang HS, Suzuma I, Miyagi M, Youngman E, Mori K, et al. Pigment epithelium-derived factor suppresses ischemia-induced retinal neovascularization and VEGF-induced migration and growth. *Invest Ophthalmol Vis Sci* 2002;**43**:821–9.
52. Mori K, Gehlbach P, Ando A, McVey D, Wei L, Campochiaro PA. Regression of ocular neovascularization in response to increased expression of pigment epithelium-derived factor. *Invest Ophthalmol Vis Sci* 2002;**43**:2428–34.
53. Shao C, Sima J, Zhang SX, Jin J, Reinach P, Wang Z, et al. Suppression of corneal neovascularization by PEDF release from human amniotic membranes. *Invest Ophthalmol Vis Sci* 2004;**45**:1758–62.
54. Jin J, Ma JX, Guan M, Yao K. Inhibition of chemical cautery-induced corneal neovascularization by topical pigment epithelium-derived factor eyedrops. *Cornea* 2010;**29**:1055–61.
55. Matsui T, Nishino Y, Maeda S, Yamagishi S. PEDF-derived peptide inhibits corneal angiogenesis by suppressing VEGF expression. *Microvasc Res* 2012;**84**:105–8.
56. Kuo CN, Yang LC, Yang CT, Lai CH, Chen MF, Chen CY, et al. Inhibition of corneal neovascularization with plasmid pigment epithelium-derived factor (p-PEDF) delivered by synthetic amphiphile INTeraction-18 (SAINT-18) vector in an experimental model of rat corneal angiogenesis. *Exp Eye Res* 2009;**89**:678–85.
57. Kuo CN, Chen CY, Chen SN, Yang LC, Lai LJ, Lai CH, et al. Inhibition of corneal neovascularization with the combination of bevacizumab and plasmid pigment epithelium-derived factor-synthetic amphiphile INTeraction-18 (p-PEDF-SAIN-18) vector in a rat corneal experimental angiogenesis model. *Int J Mol Sci* 2013;**14**:8291–305.
58. Kim WJ, Mohan RR, Mohan RR, Wilson SE. Effect of PDGF, IL-1 α , and BMP2/4 on corneal fibroblast chemotaxis: expression of the platelet-derived growth factor system in the cornea. *Invest Ophthalmol Vis Sci* 1999;**40**:1364–72.
59. Hoppenreijns VP, Pels E, Vrensen GF, Felten PC, Treffers WF. Platelet-derived growth factor: receptor expression in corneas and effects on corneal cells. *Invest Ophthalmol Vis Sci* 1993;**34**:637–49.
60. Chaoran Z, Zhirong L, Gezhi X. Combination of vascular endothelial growth factor receptor/platelet-derived growth factor receptor inhibition markedly improves the antiangiogenic efficacy for advanced stage mouse corneal neovascularization. *Graefes Arch Clin Exp Ophthalmol* 2011;**249**:1493–501.
61. Dell S, Peters S, Muther P, Kociok N, Joussen AM. The role of PDGF receptor inhibitors and PI3-kinase signaling in the pathogenesis of corneal neovascularization. *Invest Ophthalmol Vis Sci* 2006;**47**:1928–37.
62. Perez-Santonja JJ, Campos-Mollo E, Lledo-Riquelme M, Javaloy J, Alio JL. Inhibition of corneal neovascularization by topical bevacizumab (anti-VEGF) and sunitinib (anti-VEGF and anti-PDGF) in an animal model. *Am J Ophthalmol* 2010;**150**:519–28. e1.
63. Troyanovsky B, Levchenko T, Mansson G, Matvijenko O, Holmgren L. Angiotensin: an angiotensin binding protein that regulates endothelial cell migration and tube formation. *J Cell Biol* 2001;**152**:1247–54.
64. Tarui T, Miles LA, Takada Y. Specific interaction of angiotensin with integrin $\alpha(v)\beta(3)$ in endothelial cells. *J Biol Chem* 2001;**276**:39562–8.
65. Gabison E, Chang JH, Hernandez-Quintela E, Javier J, Lu PC, Ye H, et al. Anti-angiogenic role of angiotensin during corneal wound healing. *Exp Eye Res* 2004;**78**:579–89.
66. Ambati BK, Joussen AM, Ambati J, Moromizato Y, Guha C, Javaherian K, et al. Angiotensin inhibits and regresses corneal neovascularization. *Arch Ophthalmol* 2002;**120**:1063–8.
67. Chen P, Yin H, Wang Y, Wang Y, Xie L. Inhibition of VEGF expression and corneal neovascularization by shRNA targeting HIF-1 α in a mouse model of closed eye contact lens wear. *Mol Vis* 2012;**18**:864–73.
68. Mastuyugin V, Mosaed S, Bonazzi A, Dunn MW, Schwartzman ML. Corneal epithelial VEGF and cytochrome P450 4B1 expression in a rabbit model of closed eye contact lens wear. *Curr Eye Res* 2001;**23**:1–10.
69. Stoltz RA, Connors MS, Dunn MW, Schwartzman ML. Effect of metabolic inhibitors on arachidonic acid metabolism in the corneal epithelium: evidence for cytochrome P450-mediated reactions. *J Ocul Pharmacol* 1994;**10**:307–17.
70. Davis KL, Connors MS, Dunn MW, Schwartzman ML. Induction of corneal epithelial cytochrome P-450 arachidonate metabolism by contact lens wear. *Invest Ophthalmol Vis Sci* 1992;**33**:291–7.
71. Seta F, Patil K, Bellner L, Mezentsev A, Kemp R, Dunn MW, et al. Inhibition of VEGF expression and corneal neovascularization by siRNA targeting cytochrome P450 4B1. *Prostaglandins Other Lipid Mediat* 2007;**84**:116–27.
72. Enzoly A, Dunkel P, Recean Z, Gyorffy H, Toth J, Marics G, et al. Preliminary studies of the effects of vascular adhesion protein-1 inhibitors on experimental corneal neovascularization. *J Neural Transm* 2011;**118**:1065–9.
73. Nakao S, Noda K, Zandi S, Sun D, Taher M, Schering A, et al. VAP-1-mediated M2 macrophage infiltration underlies IL-1 β - but not VEGF-A-induced lymph- and angiogenesis. *Am J Pathol* 2011;**178**:1913–21.
74. Enzoly A, Marko K, Tabi T, Szoko E, Zelko R, Toth M, et al. Lack of association between VAP-1/SSAO activity and corneal neovascularization in a rabbit model. *J Neural Transm* 2013;**120**:969–75.
75. Schonherr E, Sunderkotter C, Schaefer L, Thanos S, Grassel S, Oldberg A, et al. Decorin deficiency leads to impaired angiogenesis in injured mouse cornea. *J Vasc Res* 2004;**41**:499–508.
76. Mimura T, Han KY, Onguchi T, Chang JH, Kim TI, Kojima T, et al. MT1-MMP-mediated cleavage of decorin in corneal angiogenesis. *J Vasc Res* 2009;**46**:541–50.
77. Mohan RR, Tovey JC, Sharma A, Schultz GS, Cowden JW, Tandon A. Targeted decorin gene therapy delivered with adeno-associated virus effectively retards corneal neovascularization *in vivo*. *PLoS one* 2011;**6**:e26432.
78. Zhou SY, Xie ZL, Xiao O, Yang XR, Heng BC, Sato Y. Inhibition of mouse alkali burn induced-corneal neovascularization by recombinant adenovirus encoding human vasohibin-1. *Mol Vis* 2010;**16**:1389–98.
79. Straiker AJ, Maguire G, Mackie K, Lindsey J. Localization of cannabinoid CB1 receptors in the human anterior eye and retina. *Invest Ophthalmol Vis Sci* 1999;**40**:2442–8.
80. Pisanti S, Picardi P, Prota L, Proto MC, Laezza C, McGuire PG, et al. Genetic and pharmacologic inactivation of cannabinoid CB1 receptor inhibits angiogenesis. *Blood* 2011;**117**:5541–50.
81. Saud EE, Moraes Jr HV, Marculino LG, Gomes JA, Allodi S, Miguel NC. Clinical and histopathological outcomes of subconjunctival triamcinolone injection for the treatment of acute ocular alkali burn in rabbits. *Cornea* 2012;**31**:181–7.
82. Hos D, Saban DR, Bock F, Regenfuss B, Onderka J, Masli S, et al. Suppression of inflammatory corneal lymphangiogenesis by application of topical corticosteroids. *Arch Ophthalmol* 2011;**129**:445–52.
83. Nakao S, Zandi S, Lara-Castillo N, Taher M, Ishibashi T, Hafezi-Moghadam A. Larger therapeutic window for steroid versus VEGF-A inhibitor in inflammatory angiogenesis: surprisingly similar impact on leukocyte infiltration. *Invest Ophthalmol Vis Sci* 2012;**53**:3296–302.
84. Cho YK, Uehara H, Young JR, Archer B, Ambati BK. Effect of glucocorticoid (triamcinolone acetonide) pretreatment in a murine penetrating keratoplasty and suture model. *Cornea* 2012;**31**:1468–75.
85. Zapata G, Racca L, Tau J, Berra A. Topical use of rapamycin in herpetic stromal keratitis. *Ocul Immunol Inflamm* 2012;**20**:354–9.
86. Zhao GQ, Zhang ZH, Liang T, Zhang LN, Liu JW, Du ZD. The effect of rapamycin liposome gutta inhibiting rat corneal neovascularization. *Zhonghua Yan Ke Za Zhi* 2009;**45**:146–52 [In Chinese].
87. Lee YK, Chung SK. The inhibitory effect of thalidomide analogue on corneal neovascularization in rabbits. *Cornea* 2013;**32**:1142–8.