The Ocular Surface xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

The Ocular Surface

journal homepage: www.elsevier.com/locate/jtos

Review Article

Current and emerging therapies for corneal neovascularization

Danial Roshandel[a,1], Medi Eslani[b,c,1], Alireza Baradaran-Rafii[a], Albert Y. Cheung[c], Khaliq Kurji[c], Sayena Jabbehdari[a,b], Alejandra Maiz[b], Setareh Jalali[a], Ali R. Djalilian[b,*], Edward J. Holland[c,**]

[a] Ocular Tissue Engineering Research Center, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[b] Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL, USA
[c] Cincinnati Eye Institute, Edgewood, KY/ University of Cincinnati, Department of Ophthalmology, Cincinnati, OH, USA

ARTICLE INFO

Keywords:
Eye
Cornea
Vascularization
Neovascularization
Angiogenesis
Limbal stem cell deficiency
Inflammation

ABSTRACT

The cornea is unique because of its complete avascularity. Corneal neovascularization (CNV) can result from a variety of etiologies including contact lens wear; corneal infections; and ocular surface diseases due to inflammation, chemical injury, and limbal stem cell deficiency. Management is focused primarily on the etiology and pathophysiology causing the CNV and involves medical and surgical options. Because inflammation is a key factor in the pathophysiology of CNV, corticosteroids and other anti-inflammatory medications remain the mainstay of treatment. Anti-VEGF therapies are gaining popularity to prevent CNV in a number of etiologies. Surgical options including vessel occlusion and ocular surface reconstruction are other options depending on etiology and response to medical therapy. Future therapies should provide more effective treatment options for the management of CNV.

1. Introduction

Corneal neovascularization (CNV) can result from a variety of etiologies such as contact lens wear; corneal infections; and ocular surface inflammation and injury including limbal stem cell deficiency (LSCD) [1–3]. CNV may result in decreased visual acuity from the sequelae of blood vessels invading the cornea and causing opacification of the stroma and irregularity of the corneal surface. Surface irregularity results in higher order aberrations; this can be accompanied by extravasation of fluid and lipids, which leads to corneal edema and lipid keratopathy as well as alteration of the stromal architecture [4]. These changes minimize corneal clarity and thus impede vision. In a comprehensive ophthalmology clinic, CNV was found in 4.14% of patients, of which 12% showed a decrease in visual acuity [5]. While CNV can be helpful in certain circumstances (e.g. uncontrolled corneal infections, conditions involving stromal necrosis), it is more often a pathologic consequence of various ocular surface and corneal disorders. Notably, CNV is an important risk factor for corneal graft rejection and subsequent failure [6].

1.1. Definition and terminology

The cornea is unique because it is completely avascular and alymphatic, which is essential for its clarity and optimal vision. When blood and lymphatic vessels from the pericorneal vascular plexus grow into the cornea, the result is a pathologic condition termed corneal hemangiogenesis and corneal lymphangiogenesis, respectively.

It has been proposed that the term corneal vascularization is appropriate in contrast to neovascularization, as the latter refers to a condition in which new blood vessels arise from pre-existing ones. To avoid confusion with choroidal neovascularization and due to the absence of pre-existing blood vessels in the cornea, the term “corneal vascularization” should be used for corneal vessel formation [7]. However, in reviewing the literature, majority of studies term this pathology “corneal neovascularization”. To follow prior studies in this field, the term “corneal neovascularization (CNV)” is used throughout this review.

In many pathologic conditions, lymphatic vessels grow into the cornea parallel to blood vessels. Lymphangiogenesis plays a critical role in many processes such as immunity, infection, and metastasis [8].

1.2. Vasculogenesis versus angiogenesis

Vasculogenesis comprises the *de novo* formation of vessels from vascular endothelial precursor cells (i.e. hemangioblasts and angioblasts) which are derived from mesodermal precursors (via mesodermal

* Corresponding author. 1855 W. Taylor Street, EEI, 3164, Department of Ophthalmology and Visual Sciences, University of Illinois at Chicago, Chicago, IL, 60612, USA.
** Corresponding author. Cincinnati Eye Institute/University of Cincinnati, 580 South Loop Road, Suite 200, Edgewood, KY, 41017, USA.
E-mail addresses: adjalili@uic.edu (A.R. Djalilian), eholland@holprovision.com (E.J. Holland).
[1] Drs. Roshandel and Eslani contributed equally to this paper.

https://doi.org/10.1016/j.jtos.2018.06.004
Received 22 January 2018; Received in revised form 10 June 2018; Accepted 12 June 2018

induction) [9]. In contrast, angiogenesis is a process in which endothelial cells of pre-existing vessels proliferate and form new vessels [9]. In CNV, the endothelial cells of newly formed corneal vessels originate from pre-existing limbal vessels (i.e. angiogenesis). However, pericytes, another crucial cell type in blood vessel formation, originate from bone-marrow derived precursors (i.e. vasculogenesis) [10]. Ozerdem and colleagues believe that both angiogenesis and vasculogenesis are involved in CNV and that targeting both mechanisms would be most effective in managing this condition [10]. Similar to blood vessels, lymphatic vessels may arise *de novo* from bone-marrow derived cells (i.e. CD11b-positive macrophages) or they may extend from pre-existing limbal lymphatic vessels [8,11].

2. Corneal vascular privilege

Previous studies have identified a number of mechanism(s) by which the limbal vascular plexus does not invade the cornea under normal physiologic conditions. It is believed that an imbalance between angiogenic and anti-angiogenic mechanisms in the cornea results in CNV [12].

The first proposed mechanism for CNV was proposed by Cogan, who claimed corneal swelling and subsequent disintegration of the corneal lamellae were the sole factors responsible for CNV [13]. However, further investigation revealed that corneal swelling is necessary but not sufficient for the development of CNV [14,15].

While there is no anatomical boundary between the limbal vascular plexus and the clear cornea, the angiostatic function of the limbus has been proposed as a mechanism for corneal avascularity, especially since LSCD is often associated with CNV [16–18]. It is unclear whether the limbus exerts its barrier function via a physical or functional mechanism, or both. The physical barrier effect of the limbus has been proposed by Friedenwald as a “growth pressure theory,” in which continuous self-renewal of the limbal stem cells prevents invasion of the conjunctival epithelium and subsequent vascularization of the cornea [19]. However, using a murine hemilimbal corneal injury model, Tobaigy showed factors other than the limbal barrier are involved to maintain corneal avascularity [20].

Although earlier reports supported the angiogenic properties of corneal epithelium [21,22], the predominantly anti-angiogenic role of the corneal epithelium has been widely accepted in more recent studies [23]. Clinically, the association of a persistent corneal epithelial defect (PED) with CNV and its resolution after epithelial transplantation further supports the role of corneal epithelium in preventing CNV [24].

Interestingly, the corneal epithelium releases pro-angiogenic factors such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), which are then sequestrated by the basement membrane (BM) under normal conditions [22,25]. For example, Ambati and colleagues found that the cornea contains a high quantity of VEGF-A, a potent pro-angiogenic molecule. However, it is almost completely bound to the soluble VEGF-receptor 1 (also known as soluble fms-like tyrosine kinase-1 sflt-1), thus preventing its angiogenic effects [26]. They concluded that sflt-1 is a crucial factor in corneal avascularity [26]. Ambati and colleagues have also reported that expression of sflt-1 is significantly lower in vascularized corneas (secondary to alkali burn, ocular cicatricial pemphigoid, interstitial keratitis, and aniridia) when compared to normal human corneas [27]. Inhibitory PAS (Per/Arnt/Sim) domain protein is another corneal epithelial derived factor with antiangiogenic properties, specifically against hypoxia inducible factor (HIF)/Hypoxia induced CNV [28]. In addition, VEGF receptor 3, which is constitutively expressed by the corneal epithelium, is an inhibitor of corneal angiogenesis [29].

The corneal epithelial BM also contains anti-angiogenic factors such as tissue inhibitor of metalloproteinase 3 (TIMP-3) and collagen XVIII/endostatin [30,31]. Angiostatin, restin, arrestin, endostatin, canstatin, tumstatin, thrombospondins, interleukin-1 receptor antagonist, pigment epithelial derived factor (PEDF), vasoactive intestinal peptide (VIP) and α-melanocyte stimulating hormone (α-MSH) are also anti-angiogenic molecules, which have been found in the cornea and/or the aqueous humor [4,32–34]. Given that the cornea contains both angiogenic and anti-angiogenic factors, damage to the basement membrane (BM) due to LSCD or persistent epithelial defects may result in the release of pro-angiogenic factors and loss of anti-angiogenic factors, and thus lead to CNV [35].

Several molecules with anti-lymphangiogenic properties have been identified in the cornea and aqueous humor. These include alternatively spliced VEGF receptor-2 (soluble VEGFR-2), tumor necrosis factor superfamily member 10 (Tnfsf10/Trail), tissue plasminogen activator (tPA), and thrombospondin 1 in the cornea as well as VIP and α-MSH in the aqueous humor [33,36–38].

3. Hemangiogenesis versus lymphangiogenesis

The lymphatic system is a network of vessels throughout the body that allows lymphatic fluid to return to the systemic blood circulation. In addition to lacking blood vessels, the cornea is also devoid of lymphatic vessels. The paucity of blood vessels prevents immune cells from accessing corneal antigens, and the lack of lymphatic vessels prevents cellular and cytokine traffic to the regional lymph nodes [39]. While several anti- (hem) angiogenic factors are known to be present in the normal cornea (as mentioned above), the anti- (lymph) angiogenic factors are still yet unknown. However, in vascularized human corneas, the degree of lymphangiogenesis is significantly correlated with the degree of hemangiogenesis [40,41]. In contrast, corneal lymphangiogenesis may also occur in an avascular cornea. Transient physiologic lymphangiogenesis has been reported to reduce corneal edema and increase corneal transparency in cases with corneal edema [42].

Lymphatic vessels can provide a drainage pathway for both antigenic material (cells, cellular debris) and more importantly antigen presenting cells [43,44]. Besides enabling the transport itself, lymphatic vessels enhance the speed and amount of antigenic material or antigen presenting cells that reach the regional lymph nodes [45]. This is particularly important after corneal transplantation [46]. Corneal lymphangiogenesis provides a route of exit from the graft to regional lymph nodes, which has been shown to be essential in promoting alloimmunization and subsequent graft rejection [47,48]. Corneal lymphatics in vascularized human host beds adjacent to grafted tissue could enhance the traffic of graft-derived antigens to regional lymph nodes, thereby promoting rejection [39].

4. Mechanisms of corneal neovascularization

Cogan originally described histologic evidence of a mechanism following corneal inciting injury, which led to local corneal edema accompanied by aneurysmal engorgement of venules and capillaries. After a few days, these were replaced by tiny spicule-like masses of hemorrhages in a radiating pattern which could later either form capillary channels or regress/disappear. The channels typically formed an extension in front of a pre-existing loop. With repetition of this cycle, there was progressive movement of the vessels toward the central cornea [13]. Conversely, Shi and colleagues described three pivotal steps to CNV in five models (suture-mediated, alkali injury, fungal infection, or implantation of immunogen or tumor cells) [49]. These pivotal steps included a sprout period, a vigorous stage, and a regressive stage. Although in all of the models the initial findings (corneal edema and vascular dilation) were similar to Cogan's investigations, Shi and colleagues identified more details of the events leading to CNV. First, the BM of the perilimbal capillary network is degraded by proteases released by endothelial cells. Thereafter, endothelial cells (ECs) migrate and invade the extracellular matrix (ECM) and begin to proliferate. Finally, the lumen of the new vessels forms, and the BM is remodeled [49].

The mounting evidence in literature clearly show CNV and corneal