Original article

Combined scraping, coagulation, and subconjunctival bevacizumab in corneal transplantation for bullous keratopathy with corneal neovascularization

Chun-Chi Chiang, MD, PhD, Jane-Ming Lin, MD, Yi-Yu Tsai, MD, PhD *

Department of Ophthalmology, China Medical University Hospital, Taichung, Taiwan

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A B S T R A C T

Purpose: This study investigated clinical outcomes of the combined method of scraping, coagulation, and subconjunctival bevacizumab for the treatment of corneal neovascularization (NV) in penetrating keratoplasty (PKP).

Methods: This study included patients undergoing PKP who were diagnosed with bullous keratopathy with dense subepithelial scarring that was not suitable for Descemet's stripping automated endothelial keratoplasty. Corneal NV was treated by scraping the corneal epithelium and lightly coagulating the superficial corneal stromal NV combined with subconjunctival bevacizumab injection at the end of surgery. Patients without corneal NV were used as the control group.

Results: There were six patients with vascularized corneas in the study group and three patients without vascularized corneas in the control group. The original corneal NV in the study group disappeared in all patients after surgery. Three of the six (50%) study patients experienced recurrent corneal NV. One of the three (33%) control patients developed corneal NV. These patients had no corneal NV recurrences over the next 6 months after repeat treatment. In both groups, no graft failure or chronic epithelial defects occurred.

Conclusion: The combination of scraping the corneal epithelium, coagulating the superficial corneal stromal NV and the feeding vessels in the sclera after peritomy, and subconjunctival bevacizumab injection is an effective method to treat corneal NV in corneal transplantation for bullous keratopathy.

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1. Introduction

Long-standing bullous keratopathy usually induces extensive corneal neovascularization (NV), and corneal transplantation in bullous keratopathy patients with extensive corneal NV carries a high risk of graft rejection.1,2 There are various methods available for treating corneal NV before corneal transplantation, such as argon laser photocoagulation, photodynamic therapy with different photosensitizers, and epithelial debridement.3,4 However, these therapies are often associated with thermal or mechanical damage to the cornea, which activates the inflammatory cascade and leads to an upregulation of vascular endothelial growth factor (VEGF).3 Hence, the aforementioned therapies have a high rate of vessel regeneration in the corneal stroma that is difficult to control.

Treatment using angiogenic inhibitors has become a novel method for the management of both superficial and stromal corneal NV.5–7 Bevacizumab is a full-length humanized murine monoclonal antibody against all types of VEGF. It is approved to treat metastatic colorectal cancer,8 diabetic retinopathy,9,10 choroidal NV in pathologic myopia,11 and exudative age-related macular degeneration.12–14 Recently, the effects of subconjunctival injection15–18 and topical application19–21 of bevacizumab on inhibition of corneal NV have been reported. However, in humans, bevacizumab treatment either in the form of drops22–24 or by subconjunctival administration25,26 was either ineffective or led to only partial regression of the corneal NV. With regard to patients with a previously rejected vascularized graft, bevacizumab treatment resulted in little change in the number and caliber of blood vessels.26 The lack of results in these cases was attributed to the varying levels of VEGF expression in established and actively growing vessels.26 Bevacizumab had the
largest impact on newly developed or developing vessels, and yet was ineffective against previously established blood vessels. Combined mechanical or thermal methods to destroy established NV together with subconjunctival bevacizumab to stop new vessel growth is a reasonable method to treat corneal NV. Moreover, in animal models, bevacizumab significantly inhibited NV when administered immediately after limbal injury but provided less significant regression of established NV. Therefore, destroying NV and administering bevacizumab injections should be performed simultaneously to achieve the optimal anti-neovascular effect.

Combination therapy using bevacizumab has been previously reported in three cases. Qian et al scraped the corneal epithelium and injected bevacizumab subconjunctivally in a case of peripheral ulcerative keratitis in a patient with Terrien marginal degeneration, and Gerten used argon laser coagulation and bevacizumab injections in two corneal transplants. The results revealed that this type of therapy was safe and effective.

Owing to the difficulties in performing intraoperative corneal epithelial scraping or postoperative argon laser coagulation to destroy the corneal stromal NV and the feeding vessels in the conjunctiva and sclera, we developed a method to eliminate the vessels of vascularized bullous keratopathy in corneal transplantation. We scraped the corneal epithelium in the corneal NV area and then coagulated the superficial corneal stromal NV in the peripheral cornea and the feeding vessels in the conjunctiva and sclera. After corneal transplant, we injected bevacizumab subconjuntivally. To conclude, this method of destroying and preventing corneal NV in corneal transplantation was evaluated.

2. Patients and methods

A 1-year clinical trial was designed that included all qualifying bullous keratopathy patients undergoing corneal transplantation. One doctor (Y.-Y.T.) performed all corneal transplantations in this clinical trial. We performed penetrating keratoplasty (PKP) for those with dense subepithelial scarring, which rendered them poor candidates for Descemet’s stripping automated endothelial keratoplasty (DSAEK). Patients with vascularized cornneas were included in the study group and patients without vascularized cornneas were included in the control group.

The study group underwent the following procedure: the corneal epithelium in the corneal NV area was scraped and the superficial corneal stromal NV in the peripheral cornea was lightly coagulated. After partial peritomy, the feeding vessels of all corneal stromal NV in the sclera were coagulated. During corneal and scleral coagulation, the limbus was spared. If the corneal epithelial NV was extensive, we coagulated the epithelial NV to decrease hemorrhage before scraping the corneal epithelium in the corneal NV area. In the control group, the corneal epithelium was kept intact. At the end of surgery, 0.3 mL subconjunctival bevacizumab injection was administered to the study group.

After corneal transplantation, all patients stayed in the hospital for 4–5 days and were followed up at 1, 2, 3, 4, 6, and 8 weeks and 3, 4, 5, and 6 months postoperatively. After 6 months, follow-up examinations were performed every 2–3 months.

At each visit, two digital corneal photographs were taken using a Topcon DC-1 digital camera attached to the slit-lamp microscope. The photographs were graded by one observer for extent, centricity, and density of corneal vascularization (as previously described by Bahar et al). Briefly, extent was defined according to the number of clock hours affected by NV (score 1–12). Centricity was defined as the distance the new vessels extended from the limbus towards the visual axis. Density was graded 1–4 according to the density of NV. Each picture was scored by a second investigator according to the changes in NV extent, centricity, and density before and after surgery.

During follow-up, if NV was invading the cornea by more than 1 mm, the patient received another treatment of epithelial scraping, NV coagulation, and 0.3 mL subconjunctival bevacizumab injection. Efficacy of the anti-corneal NV method was evaluated as the recurrence rate of corneal NV and the number of times the patients required retreatment during the postoperative 6 months.

In accordance with the Declaration of Helsinki, the experimental status of the method and off-label use of bevacizumab was explained to all patients, following which, informed patient consent was obtained. Study protocols were approved by an institutional review board of the China Medical University Hospital.

3. Results

There were nine bullous keratopathy patients included in this clinical trial. In the study group, there were six patients with vascularized cornneas and in the control group there were three patients without vascularized cornneas. Among the six patients in the study group, there were five failed PKPs and one pseudophakic bullous keratopathy (PBK). Patient characteristics and average scores preoperatively and postoperatively are summarized in Table 1. There were two PBKs and one failed deep anterior lamellar keratoplasty in the control group. All of the original corneal NV in the study group disappeared after surgery (Fig. 1). However, three of the six (50%) study patients had recurrent corneal NV. Among the recurrent patients, one occurred at the 6th week and two at the 8th week of the study. They underwent repeat epithelial scraping, coagulation, and 0.3 mL subconjunctival bevacizumab injection. After the booster, there were no further recurrences of corneal NV in 6 months; however, one of the three patients had an episode of acute rejection 3 weeks after the booster. The rejection subsided after topical steroid treatment. One of the three (33%) controls had corneal NV 2 weeks after PKP and needed treatment of epithelial scraping, coagulation, and 0.3 mL subconjunctival bevacizumab injection. The patient had no corneal NV recurrences over the next 6 months. Neither the study nor the control group had graft failure or chronic epithelial defects.

### Table 1

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Age</th>
<th>Sex</th>
<th>Disease</th>
<th>Preoperative</th>
<th>No of injection</th>
<th>Postoperative 12 months</th>
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</thead>
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<tr>
<td></td>
<td>Extent</td>
<td>Centricity</td>
<td>Density</td>
<td>Extent</td>
<td>Centricity</td>
<td>Density</td>
</tr>
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<td>M</td>
<td>Failed PKP</td>
<td>6</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>2</td>
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<td>F</td>
<td>PBK</td>
<td>4</td>
<td>1.5</td>
<td>1</td>
</tr>
<tr>
<td>3</td>
<td>34</td>
<td>F</td>
<td>Failed PKP</td>
<td>4</td>
<td>1.5</td>
<td>2</td>
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<tr>
<td>4</td>
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<td>M</td>
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<td>5</td>
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<td>1</td>
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<tr>
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<td>M</td>
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<td>2</td>
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<tr>
<td>6</td>
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<td>M</td>
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<td>1.5</td>
<td>1</td>
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</table>
4. Discussion

Before the application of anti-VEGF therapy, various surgical and medical treatments were used to counter corneal NV. Unfortunately, these treatments did not provide good control of vessel progression and recurrence. Surgery often activates biological mediators of the inflammatory cascade and this leads to an upregulation of VEGF, consequently, such therapies have an immediate positive effect but are associated with a high rate of vessel recanalization. Among medical treatments available, steroids remain the treatment of choice to control corneal NV. However, steroids alone are not always effective and can contribute to adverse effects, including glaucoma, infection, and cataract formation.

Bevacizumab has recently been compared to betamethasone, as bevacizumab is reported to be effective in the prevention of formation as well as the regression of major vessels. However, the most prominent effect of bevacizumab is on newly developed or developing vessels and it is relatively ineffective against previously established blood vessels. Because corneal NV is a chronic and progressive phenomenon and vessels found at the time of diagnosis are a mixture of new and old vessels, a combination of surgical methods in addition to bevacizumab therapy is a reasonable treatment modality; surgery removes mature vessels, whereas bevacizumab prevents new vessels from growing.

Moreover, corneal NV is often associated with epithelial and stromal NV, thus removing the corneal epithelial NV and destroying the corneal stromal NV and the feeding vessels in the conjunctiva and sclera is often necessary. Surgical treatments by argon laser photocoagulation or epithelial scraping are not sufficient for the removal of stromal NV and feeding vessels in the conjunctiva and sclera. For these reasons, we scraped the epithelium, coagulated the superficial corneal stromal NV, and coagulated the feeding vessels in the sclera after peritomy. Furthermore, the epithelial defect after scraping the epithelium allows bevacizumab to easily penetrate the former NV area.

Bevacizumab can be used both subconjunctivally and topically and both routes are reported to exert partial effects; however, topical application of bevacizumab was found to cause spontaneous loss of corneal epithelial integrity and progression of stromal thinning. There were no corneal problems reported with subconjunctival bevacizumab use, and thus subconjunctival bevacizumab may be a safer option than topical bevacizumab, particularly in corneal transplantation.

In our study, half of the patients in the PKP study group needed two rounds of scraping, coagulation, and subconjunctival bevacizumab injection. We hypothesized two explanations for this. Firstly, there were ocular surface complications after PKP, such as changes in the corneal curvature and tear distribution or stitch-related inflammation. Secondly, various corneal conditions may affect the number of injections required. Long-term bullous keratopathy results in recurrent corneal epithelial defects, corneal NV, and subepithelial haze or scar. In bullous keratopathy patients, we performed PKP only on those with a dense subepithelial scar. A dense subepithelial scar usually accompanies severe corneal NV, which may indicate that the cornea requires a larger dose of bevacizumab than the 0.3-mL dose used. The three controls in the PKP group were included because they had bullous keratopathy with a dense subepithelial scar rarely found without corneal NV.

Of the three PKP study patients with recurrent corneal NV, one experienced graft rejection even though additional therapy was performed and there was no apparent NV growth after the second round of therapy. This may be due to the fact that there is only a small evident part of corneal NV invasion with slit-lamp...
examination; the first recurrent NV may have reached the graft and triggered the immune system prior to a 1-mm invasion of the cornea. Hence, when performing PKP in bullous keratopathy patients with intensive corneal NV, a dose >0.3 mL bevacizumab injection intraoperatively or an early booster prior to 1-mm corneal invasion postoperatively may be needed because recurrence of corneal NV will result in a higher chance of rejection.

Theoretically, a control group for this study should comprise patients with bullous keratopathy with corneal NV who are not treated with combined scraping, coagulation, and subconjunctival bevacizumab injection. However, this is not ethically feasible as corneal transplantation in these patients without removing corneal NV carries a high risk of graft rejection. Hence, we chose bullous keratopathy patients without corneal NV as control subjects.

In conclusion, the combined method described in this study might be an effective alternative to treat corneal NV in PKP for bullous keratopathy; however, repeated injections of bevacizumab and long-term follow-up may be needed to validate our study.

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