Amniotic Membrane Transplantation in Infectious Corneal Ulcer

Jae-Soon Kim, M.D., Jae-Chan Kim, M.D., Tae-Won Hahn, M.D., and Woo-Chan Park, M.D.

Purpose. To evaluate the efficacy of amniotic membrane transplantation in the management of treated infectious corneal ulcer in which inflammatory reactions were responsible for corneal damage. Method. A prospective study of 21 consecutive eyes (21 patients) was performed. Sufficient antibacterial, antifungal, or antiviral agents were applied to eradicate causative organisms before permanent or temporary amniotic membrane transplantation, or a combination of the two in few patients. The amniotic membrane was soaked in antiinfective agents before transplantation in all cases. Results. After amniotic membrane transplantation, follow-up times ranged from 4 to 28 months (mean, 18 months). Clinical indications included *Staphylococcus* species (four cases), *Pseudomonas* species (five cases), *Acanthamoeba* species (three cases), fungus (two cases), and herpesvirus (seven cases). The corneal surface was healed successfully and recurrences of microbial infection were not noted in any case. Visual acuity was improved in cases that were nonscarring or after additional penetrating keratoplasty. Conclusion. Amniotic membrane transplantation seems to be a useful adjunctive surgical procedure for the management of infectious corneal ulcer by promoting wound healing and reducing inflammation. Key Words: Amniotic membrane—Infectious keratitis—Wound healing.

Most corneal epithelial defects heal without complications because of an integrated ocular surface defense. In the presence of compromised ocular surface defense, such as malfunction of lids or tear film, nerve damage, and corneal infection, chronic inflammation may lead to persistent epithelial defects and stromal melting. Conventional treatments of such defects aim to eliminate the underlying diseases and restore the healthy corneal surface.

Microbial keratitis, caused by bacteria, fungi, viruses, or parasites, is a potentially sight-threatening ocular infection resulting in permanent corneal opacity or persistent epithelial defect. Based on laboratory studies and knowledge of the pathogen, a therapeutic plan is initiated. To this date, remarkable progress has been made concerning the mechanism of wound healing and the involvement of various growth factors to facilitate corneal wound healing. For example, in corneal neurotrophic ulcer, nerve growth factor would prompt healing and restoration of visual sensitivity. In addition, various surgical procedures, such as conjunctival flap, have been proposed for corneal surface reconstruction. Only a few of them have been widely used.

One of the principal objectives of therapy for infectious keratitis is to prevent tissue destruction and irreversible corneal structural alterations. Injured corneal epithelium, infiltrating neutrophils, and some infectious organisms lead to production of various enzymes that contribute to stromal keratolysis. Collagenase is also produced by host corneal tissue. Several adjunctive modalities have been suggested to reduce the destructive effects of various enzymes released as a result of progressive infectious keratitis. Enzyme inhibitors, including disodium edetate (ethylenediaminetetraacetic acid [EDTA 0.05 M], acetylcysteine, heparin 2%, and garlardin (synthetic matrix metalloproteinase [MMP] inhibitor), have been shown experimentally to be effective.

After the development by Kim and Tseng in 1995 of the modern method of using preserved amniotic membrane, Kim and Lee proposed the use of human amniotic membrane for the treatment of epithelial defects with corneal ulcers. Recently, Tseng et al. reported that neurotrophic persistent ulcers can be treated successfully with amniotic membrane transplantation. These reports were postinfectious that might have the underlying causes of herpes simplex virus and herpes zoster virus, but the infection was rather remote. Therefore, in infectious keratitis, it remains unclear whether surgical reconstruction of the corneal surface should be preformed soon because of the concern of recurrence of infection.

Amniotic membrane consists of a thick basement membrane and an avascular stroma that contains a high concentration of basic fibroblast growth factor, basement membrane components, and unknown trophic factors. In a previous study, we reported that the amniotic membrane contains various forms of proteinase inhibitors and that the stromal matrix of the amniotic membrane has the ability to exclude inflammatory cells. In addition, amniotic membrane transplantation could promote healing and inhibits proteinase activity on wound healing after acute corneal alkali burn. These effects would directly and indirectly modulate the inflammatory process induced by infection. The purpose of this study was to evaluate the efficacy of amniotic membrane transplantation in patients with infectious corneal ulcers.

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PATIENTS AND METHODS

Patient Characteristics
Between November 1997 and September 1999, amniotic membrane transplantation was performed in a prospective manner on 21 eyes of 21 consecutive patients between the ages of 18 and 77 years. All patients had clinical and microbiologic signs of an acute corneal ulceration induced by infection and were referred to the Departments of Ophthalmology of Chung-Ang University, Catholic University, and Dong-A University by different physicians. The causative organisms of 21 corneal ulcers were *Staphylococcus* species (n = 4), *Pseudomonas* species (n = 5), *Acanthamoeba* species (n = 3), fungus (n = 2), and herpesvirus (n = 7). All the procedures followed the tenets of the Declaration of Helsinki. Informed consent was obtained after explanation of the nature of the possible consequence of the treatment method.

Preparation of Amniotic Membrane
Human amniotic membrane was prepared and preserved by a previously described method with minor modifications. In brief, human placenta was obtained shortly after elective cesarean deliveries. HIV, human hepatitis types B and C, and syphilis had been excluded by serologic tests. Under a lamellar flow hood, the placenta was cleaned of blood clots with sterile saline solution containing 50 μg/mL of neomycin, 100 μg/mL of gentamicin, 100 μg/mL of amphotericin B, and 2.5 μg/mL of amphotericin B. The amniotic membrane was separated from the remaining chorion by blunt dissection and flattened onto a nitrocellulose membrane with the epithelium–basement membrane surface up. The paper with the adherent amniotic membrane was stored at −70°C in a sterile vial containing DMEM (Dulbecco’s Modified Eagle Medium, Gibco, CA, U.S.A.) and glycerol in a ratio of 7:3 (vol/vol) before transplantation.

Surgical Technique
As a routine, the standard microbiologic workup included smears and cultures to identify the pathogen. Patients were initially treated with broad-spectrum antibiotics. Once the pathogen was identified, we administered specific antiinfective topical treatment of an appropriate period. When we thought the activity of the pathogen was suppressed and improvement of the lesion was apparent, amniotic membrane was soaked in antiinfective agents and then transplanted onto corneal lesions in all cases. Amniotic membrane transplantation was divided largely into two methods depending on treatment purpose and condition. For the purpose of improving the microenvironment of limbal epithelial stem cells and corneal epithelial cells that were adjacent to a superficial corneal ulcer and if there was a great likelihood to improve the visual outcome, temporary amniotic membrane patching with the epithelium side down was performed to cover the entire cornea by suturing it onto the bulbar conjunctiva with 10-0 nylon if a widespread corneal lesion was present. After 3 days, the temporary amniotic membrane patching was removed in all cases.

### TABLE 1. Demographic, clinical, and surgical data on 21 patients with Amniotic Membrane Transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age (y)</th>
<th>Diagnosis</th>
<th>VA Findings</th>
<th>Medication</th>
<th>Procedure</th>
<th>Postoperative</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>F/77</td>
<td>Pseudomonal keratitis, RE</td>
<td>LP</td>
<td>Paracentral corneal melting and opacity with severe necrotic infiltration</td>
<td>Fortified tobramycin, cefuroxime</td>
<td>2 1 Debridement</td>
<td>LP</td>
</tr>
<tr>
<td>2</td>
<td>M/22</td>
<td>Pseudomonal keratitis, RE</td>
<td>10/20</td>
<td>Central corneal melting</td>
<td>Fortified tobramycin, ciprofloxacin</td>
<td>1 — —</td>
<td>18/20 Decreased size of epithelial defect</td>
</tr>
<tr>
<td>3</td>
<td>F/38</td>
<td>Pseudomonal keratitis, LE</td>
<td>HM</td>
<td>Central stromal melting</td>
<td>Fortified vancomycin, amikin</td>
<td>2 — —</td>
<td>8/20 Completely healed epithelium Complete healing with slight corneal opacity</td>
</tr>
<tr>
<td>4</td>
<td>F/18</td>
<td>Pseudomonal keratitis, RE</td>
<td>HM</td>
<td>Central ulceration</td>
<td>Fortified tobramycin, ciprofloxacin</td>
<td>1 — —</td>
<td>18/20</td>
</tr>
<tr>
<td>5</td>
<td>M/63</td>
<td>Pseudomonal keratitis, RE</td>
<td>HM</td>
<td>Central necrosis with microleakage and neovascularization</td>
<td>Oftoxacin, fluromethalone</td>
<td>— 1 SCL 30 cm</td>
<td>Decreased neovascularization, no leakage</td>
</tr>
<tr>
<td>6</td>
<td>M/44</td>
<td>Staphylococcal keratitis, RE</td>
<td>3/20</td>
<td>Geographic staining and SPK</td>
<td>Ciprofloxacin</td>
<td>1 — SCL 18/20</td>
<td>Completely healed epithelium Occurred at post-LASIK 1 month</td>
</tr>
<tr>
<td>7</td>
<td>M/59</td>
<td>Staphylococcal keratitis, LE</td>
<td>HM</td>
<td>Paracentral necrotic infiltration with impending perforation</td>
<td>Ciprofloxacin, fortified vancomycin</td>
<td>1 1 Debridement</td>
<td>4/200</td>
</tr>
<tr>
<td>8</td>
<td>M/23</td>
<td>Staphylococcal keratitis, LE</td>
<td>HM</td>
<td>Central ulceration with PED</td>
<td>Cefotaxol, gentamicin</td>
<td>1 — SCL 20/20</td>
<td>Complete healing</td>
</tr>
</tbody>
</table>

TAMP indicates temporary amniotic membrane patching; PAMG, permanent amniotic membrane graft; VA, visual acuity; HM, hand movement; LP, light perception; FC, finger count; RE, right eye; LE, left eye; SCL, bandage contact lens; M, male; F, female.
Repeat temporary amniotic membrane patching was performed if necessary.

When the primary purpose of treatment was to secure the corneal surface and to prevent corneal perforation rather than to improve the visual outcome as the ulceration was deep into the corneal stroma, permanent amniotic membrane graft was performed by suturing the amniotic membrane permanently as a graft with the stromal surface facing the ulcer bed as single or multiple layer. Also in five cases, a therapeutic soft contact lens was applied to facilitate corneal epithelial healing after amniotic membrane transplantation. In six cases, combined amniotic membrane transplantation was performed.

RESULTS

The patients’ preoperative and postoperative visual acuity and other pertinent clinical information, such as history of surgery and other abnormal findings, are shown in Tables 1–3. After amniotic membrane transplantation, the follow-up period ranged from 4 to 28 months (mean, 18 months). We did not observe adverse effects induced by transplanted amniotic membrane, such as recurrence of infection or superinfection on ulcerated lesion. On the contrary, there was a consistent reduction in ocular inflammation within the first few days of surgery, and ulcerative wounds were healed completely in all patients. This effect was reflected by a significant subjective improvement of symptoms by the patients.

Visual acuity increased in all successful cases, except for cases 3, 4, 6, 18, and 21 because of irreversible corneal opacity. Of these five patients, two underwent subsequent penetrating keratoplasty and their visual acuity improved to 20/20 and 14/20. In case 4, amniotic membrane transplantation prevented corneal perforation successfully by applying multilayered permanent amniotic membrane graft.

Case 1: Pseudomonal Keratitis

A 77-year-old woman had pain in her right eye for 1 week. Visual acuity was light perception. On examination there was a

<table>
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<th>TABLE 2. Demographic, clinical, and surgical data on 21 patients with Amniotic Membrane Transplantation</th>
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<tbody>
<tr>
<td>Patient</td>
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<tr>
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<tr>
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<td>14</td>
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<td>15</td>
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<td>16</td>
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</tbody>
</table>

TAMP, temporary amniotic membrane patching; PAMG, permanent amniotic membrane graft; VA, visual acuity; HM, hand movement; LP, light perception; FC, finger count; RE, right eye; LE, left eye; SCL, bandage contact lens; M, male; F, female.
paracentral ulcer with severe necrotic infiltration (Figs. 1A and 1B). Hospital cultures and smears were consistent with *Pseudomonas aeruginosa*. The patient was prescribed fortified tobramycin and cefuroxime topical drops. Within 5 days of treatment, the abscess progressed to 60% corneal thinning. Twenty days after admission, permanent amniotic membrane graft and temporary amniotic membrane patching were performed, with the former just covering the ulcer lesion and the latter covering the entire corneal surface. Ten days later, examination showed a completely healed epithelium. The follow-up examination after 2 months showed a stable corneal surface and quiescent scar (Figs. 1C and 1D).

**Case 10: Acanthamoeba Keratitis**

An 18-year-old man had blurred vision in his left eye and visual acuity of hand motions after soft contact lens wear for 24 hours after washing it with city water. The referring ophthalmologist had prescribed fortified tobramycin and cefazolin, and the results of corneal scraping for culture and smears were negative. Hospital admission cultures and smears were also negative. On initial examination, a large central ulceration with an irregular surface and whitish ring infiltration was observed (Figs. 2A and 2B).

Corneal biopsy was performed and showed *Acanthamoeba castellani*. The patient was prescribed ciprofloxacin, 0.02% polyhexamethylene biguanide, and Neosporin (GlaxoWellcome, England) topical drops. Oral itraconazole (400 mg/d) was added. During admission, corneal thinning progressed, and temporary amniotic membrane patching (five times) and permanent amniotic membrane graft (one time) were performed. The same medication was continued after surgery. Two months after admission, the corneal surface was healed with opacity and infiltration (Figs. 2C and 2D). The visual acuity remained at hand motions. Fourteen days after permanent amniotic membrane graft, penetrating keratoplasty was indicated.

### TABLE 3. Demographic, clinical, and surgical data on 21 patients with Amniotic Membrane Transplantation

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex/age (y)</th>
<th>Diagnosis</th>
<th>Preoperative</th>
<th>Procedure</th>
<th>Postoperative</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>17</td>
<td>M/55</td>
<td>Persistent herpetic keratitis, LE</td>
<td>HM Stromal melting with persistent, epithelial defect</td>
<td>TFT</td>
<td>1 — —</td>
<td>20/50 Completely healed epithelium, mixed corneal opacity</td>
</tr>
<tr>
<td>18</td>
<td>M/49</td>
<td>Herpetic keratitis, LE</td>
<td>2/20 Central superficial ulceration</td>
<td>Acyclovir, ciprofloxacin</td>
<td>1 — —</td>
<td>14/20 Complete healed epithelium with decreased corneal opacity</td>
</tr>
<tr>
<td>19</td>
<td>M/66</td>
<td>Herpetic keratitis, LE</td>
<td>HM Hypertrophic ulceration with corneal opacity</td>
<td>Acyclovir, hyalouronate</td>
<td>1 1 SCL</td>
<td>HM Decreased epithelial defect with corneal opacity</td>
</tr>
<tr>
<td>20</td>
<td>F/45</td>
<td>Herpetic keratitis, RE</td>
<td>6/20 Geographic epithelial defect</td>
<td>Acyclovir, hyalouronate</td>
<td>1 — —</td>
<td>8/20 Complete healed epithelium</td>
</tr>
<tr>
<td>21</td>
<td>M/66</td>
<td>Herpetic keratitis, RE</td>
<td>8/20 Multiple punctate erosion with ulceration</td>
<td>Acyclovir, TFT, ciprofloxacin</td>
<td>1 — SCL</td>
<td>20/20 Completely healed epithelium</td>
</tr>
</tbody>
</table>

TAMP, temporary amniotic membrane patching; PAMG, permanent amniotic membrane graft; VA, visual acuity; HM, hand movement; LP, light perception; FC, finger count; RE, right eye; LE, left eye; SCL, bandage contact lens; M, male; F, female.

**FIG. 1.** Case 1. A and B: Slit-lamp biomicroscopic examination on admission showed paracentral ulceration with severe necrotic infiltration. C: Ten days after surgery, a high-power view with direct illumination showed that two layered amniotic membranes were covered ulcerative lesion and healthy cornea. The epithelial covering on the amniotic membrane graft was entirely completed without inflammation. D: Fluorescence staining indicated a stable epithelium.
toplasty was performed. Follow-up examination 3 months after penetrating keratoplasty showed a well-healed corneal surface and 20/20 visual acuity.

Case 13: Aspergillus Keratitis

A 72-year-old woman had pain, a headache, and blurred vision in her left eye after ocular trauma. Visual acuity was light perception. The referring ophthalmologist had performed corneal scrapings that showed *Aspergillus fumigatus*. Ocular examination showed a hazy stroma with an epithelial defect, hypopyon, and keratic precipitates (Fig. 3A). The patient was prescribed 2% fluconazole and natamycin topical drops with oral itraconazole (400 mg/d). Ten days after admission, debridement of the corneal wound, temporary amniotic membrane patching, and permanent amniotic membrane graft were performed. During surgery, we noted 80% corneal thinning. Two weeks after surgery, the patient had no pain, and the examination showed no epithelial defect or active lesion (Figs. 3B and 3C). Two months after surgery, penetrating keratoplasty was performed and 2 months later, the patient had a stable corneal surface and 14/20 visual acuity with correction.

DISCUSSION

Based on the observation made by Brown, who used the rabbit peritoneum to promote healing and prevent the spread of necrosis in the acutely burned ocular surface, Sorsby et al. used chemically processed dry amniotic membrane as a patch for treating acute ocular burns. For reasons still unclear, the use of amniotic membrane disappeared from the literature. Most recently, several reports announced the uses of amniotic membrane in transplantation for reconstruction of various ocular surfaces damaged by disease. Our recent investigation showed that amniotic membrane contains several proteinase inhibitors, such as α1-antichymotrypsin, α2-macroglobulin, α1-antitrypsin, α2-antiplasmin, and inter-α1-trypsin inhibitor. In addition, the amniotic membrane protein extracts were shown to have inhibitory effects on various proteases, such as elastase and protease in polymorphonuclear leukocytes and serine protease from *A. castellani* in vitro.

This study noted successful corneal surface reconstruction by amniotic membrane transplantation and long-term stability of the corneal epithelium in all patients. The initial and consistent finding in this study was a reduction in the ocular inflammation in the early phase after amniotic membrane transplantation. Before the application of amniotic membrane transplantation, aggressive clinical and microbiologic workups of an acute corneal ulceration induced by infection was considered. Thereafter, effective and sufficient antiinfective drug treatment, such as antibiotics and antiviral or antifungal agents, was performed for an appropriate period of time. It was known that within 48 to 72 hours of appropriate and effective bactericidal therapy, the progression of keratitis is halted in bacteria-induced corneal ulceration. Therefore, when the activity of the pathogen is suppressed and clinical signs indicate improvement of the lesion, amniotic membrane transplantation can then be applied safely and effectively.

Pathogenic microorganisms are known to produce disease in the cornea by directly invading the host tissue, by secreting various metabolic products (enzymes, acids, and toxins), or by a combination of both methods. For example, the extensive corneal destruction caused by *P. aeruginosa* has been shown to be mediated by proteolytic enzymes. It is suspected that extracellular proteinase mediates the invasion of the corneal tissue by the microorganism and causes the corneal melting and ulceration seen in many cases. Although the microorganism has been shown to release collagenase, however, it is generally thought that most of this proteolytic enzyme is endogenously released by epithelial and stromal cells and accumulated by polymorphonuclear leukocytes. A series of MMPs also play an important role in corneal wound healing and pathologic conditions of infectious keratitis. Infectious ulcerative keratitis may be modified by controlling the MMP activity in the tissue. MMP-2 and MMP-9 have a capacity to degrade basement membrane collagens (types IV and VII). Syn-
In addition, amniotic membrane may have additional effects. The amniotic membrane, soaked in antinfective agent before application, would have a long-term drug delivery effect. We noted that amniotic membrane, soaked in ofloxacin before application, showed a higher concentration, like that of the collagen shield, than topical application after 1 and 2 hours (data not shown). Amniotic membrane transplantation therefore may be useful to reduce the frequency of the standard treatment with topical eye-drops, especially in the early period of an infectious corneal ulcer. Recent evidence has shown that amniotic membranes contain cystatin E, an analogue of cysteine proteinase inhibitor. This may explain why amniotic membrane transplantation is useful in herpetic keratitis because cystatin E has complementary antiviral properties. We have also found that the amniotic membrane protein contains heat shock protein that has a well-known cytoprotective function (data not shown). These heat shock proteins might prevent corneal cell damage from oxidative stress and tumor necrosis factor-α.

In conclusion, we have found that amniotic membrane transplantation therapy represents a viable method of treatment to promote healing and prevent progressive melting of refractory corneal ulcers induced by infectious keratitis. We thus argue that it might be considered a first-line surgical technique when maximal medical treatment has failed.

REFERENCES