Use of bovine pericardium (Tutopatch®) graft for surgical repair of deep melting corneal ulcers in dogs and corneal sequestra in cats

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Abstract

Objective To evaluate the efficacy of bovine pericardium (BP) graft in the treatment of deep melting corneal ulcers in three dogs and corneal sequestra in three cats.

Procedure Three dogs with keratomalacia affecting the deep third of the stroma and three cats with corneal sequestrum were evaluated and underwent surgery. Following keratectomy, BP material was placed into the keratectomy bed and sutured to the recipient cornea with 9/0 polyglactin suture material. Postoperative treatment with topical and systemic antibiotics, systemic nonsteroidal anti-inflammatory agents, and topical atropine was prescribed. Follow-up examinations were carried out 1, 2 weeks, 1 and 2 months after the surgery and consisted of a complete ophthalmic examination. Optical coherence tomography (OCT) was performed 1 and 2 months after the surgery in one dog and in one cat.

Results At 1 week, corneal neovascularization was present around the BP graft in all cases. Four weeks after the BP graft, in two dogs and in all cats, the vascularization was regressing and the graft was integrated into the cornea, which was regaining transparency. Topical treatment with anti-inflammatory agents was then prescribed for 2 weeks. Two months after the surgery, 5 of 6 corneas in two dogs and three cats had healed with focal corneal scarring. The remaining dog had progression of the keratomalacia involving the deep BP graft that required additional surgery, but became blind.

Conclusion Bovine pericardium graft offers a promising option for surgical reconstruction of the cornea following keratectomy for the management of corneal ulcers and sequestra.

Key Words: cat, cornea, dog, pericardium, sequestrum, ulcer

INTRODUCTION

Corneal ulcers are frequently traumatic in origin. Secondary bacterial infection is common. The normal corneal healing process requires the production of proteases and collagenases. They aim at removing devitalized cells and debris from the cornea. Corneal epithelial cells, fibroblasts, and some bacteria and fungi produce these proteolytic enzymes, which may have detrimental effects on the corneal stroma. The progressive dissolution of the corneal stroma eventually leads to keratomalacia. These ‘melting’ ulcers may be unresponsive to aggressive medial therapy and require specific surgical procedures.1

Corneal sequestrum is a common disorder in cats,2 particularly in the Persian and Himalayan breeds. It is rarely seen in dogs3 and in horses.4 In cats, it has been associated with feline herpes virus 1 (FHV-1) or chronic corneal irritation by physical factors such as entropion or distichiasis.2,5–8 This condition appears as a brown to black lesion of corneal necrosis of 1–2 mm in diameter to more than half of the cornea in size. It may be associated with intense corneal neovascularization. Ocular pain ranges from none to marked.2 Debridement of necrotic corneal tissue by keratectomy facilitates resolution.2,6,9–11 If the keratectomy is deep, a graft may be necessary.2

The goal of treatment of corneal disease is to restore structural integrity with minimal alteration in corneal transparency. Many surgical techniques have been described for the management of keratomalacia and sequestrum: keratectomy combined with conjunctival...
grants, corneal conjunctival transposition, porcine small intestinal submucosa graft, amniotic membrane transplantation, and heterologous penetrating keratoplasty.

Over the past decades, there has been a growing interest in the use of bovine pericardial patch in the surgical management of many pathologic conditions in humans including carotid dissection, cardiac defects, or tracheal rupture. In human ophthalmology, several studies have reported the use of BP in the management of corneal wounds or as a wrapping material for hydroxyapatite orbital implants. In veterinary ophthalmology, Barros et al. described the use of equine pericardium as a keratoprosthesis in the dog. In their reports, equine pericardium was successfully used for the surgical treatment of experimentally created superficial keratectomy and of corneo-scleral reconstruction after a traumatic perforation or the removal of an epibulbar melanoma in two dogs.

The purpose of the present study was to evaluate the use of BP for the repair of keratomalacic corneal ulcers in dogs and corneal sequestra in cats.

MATERIAL AND METHODS

Three dogs with keratomalacia affecting the deep third of the cornea and three cats with corneal sequestra were included in this study. All animals were adults between 2 and 9 years of age for dogs and 4 and 8 years for cats.

Each animal underwent a complete ophthalmic examination: Schirmer tear test, slit-lamp biomicroscopy, and intraocular pressure measurement by rebound tonometry. A Seidel test was performed in the dogs.

In all three dogs, the melting corneal ulcer was present paracentrally, without corneal vascularization. It affected the deep third of the cornea (Fig. 1). The Seidel test was negative in all animals. Physical examination was normal. The owners of Dogs 1 and 2 reported ocular trauma 3 days before referral. All dogs had been treated with topical and/or systemic antibiotics and topical acetyl cysteine. The clinical data of each animal are presented in Table 1.

In all three cats, the sequestrum was central and associated with peripheral neovascularization (Fig. 2). By history, the sequestrum was accompanied by a 3-month history of ocular disease in Cat 1 and several weeks in Cats 2 and 3. The sequestrum was well tolerated in Cat 1 and associated with a moderate level of pain in Cats 2 and 3. Physical examination was normal in all three cats. Polymerase chain reaction testing for FHV-1 was performed in all cats, and the result was positive only for Cat 1. Treatment prior to surgery included topical and/or systemic antibiotics, topical acetyl cysteine, and/or topical antiviral agents.

Surgical procedure

General anesthesia was induced by intravenous injection of medetomidine (Domitor; Janssen, Issy-les-Moulineaux, France) 2 μg/kg, and ketamine (Kétamine Virbac; Virbac, Carros, France) 5 mg/kg, followed by isoflurane gas (Isoflurane Belamont; Nicholas Piramal, London, UK) inhalation. The affected eyes were routinely prepared for ocular surgery with 5% povidone iodine solution antisepsis.

In the dogs, keratectomy to remove necrotic and collagenolytic tissue was performed using curved, blunt-tip Castroviejo scissors. In all dogs, the surgically removed cornea was submitted for bacterial culture.

In the cats, a corneal trephine was used to demarcate unhealthy cornea around the lesion, which was excised with sharp dissection.

The 40 × 50 × 0.1 mm plaque of sterile BP (Tutopatch; Tutogen, Metz, France) was removed from its package and trimmed with a corneal trephine to obtain a circular graft of the same diameter as the keratectomy defect. According to the manufacturer’s recommendations, the smooth side was marked with a corneal marking pen prior to rehydration in a solution of gentamicin 4 mg/mL (G4; Virbac) for 1 min. The graft was then placed over the keratectomy bed, the rough side in contact with the stroma. Then, it was fixed to the healthy cornea with a simple interrupted, simple continuous, or Ford interlocking continuous suture pattern of 9/0 polyglactin 910 (Vicryl monofil absorbable suture; Ethicon, Janssen, Noisy-le-grand, France) (Figs 3 and 4). An Elizabethan collar was recommended for 1 week postoperatively.

Postoperative treatment consisted of topical administration of tobramycin eye drops (Tobrex; Alcon, Rueil-Malmaison, France) three times daily for 4 weeks and atropine 1% eye drops (Atropine Faure 1%, Paris, France) three times the day after surgery and then once daily for three additional days. Each animal received oral amoxicillin and clavulanic acid (Synulox; Pfizer, Paris, France) 12.5 mg/kg twice daily for 10 days and oral tolfenamic acid (Tolfedine; Vetoquinol, Lure, France) 4 mg/kg once daily for 1 week.

Figure 1. Dog 1: melting corneal ulcer located at the axial cornea.
Follow-up examinations were performed 1, 2, 4, and 8 weeks following surgery and consisted of slit-lamp biomicroscopy, IOP measurement, and indirect ophthalmoscopy. OCT (Opko®; EDC-Lamy, Carvin, France) was performed 4 and 8 weeks after the surgery in Dog 1 and Cat 3 to assess the BP graft interface and assimilation by the cornea.

Table 1. Clinical data and outcome of dogs with melting corneal ulcers and cats with corneal sequestra treated with bovine pericardium (BP) grafts

<table>
<thead>
<tr>
<th>Case</th>
<th>Breed</th>
<th>Age/gender</th>
<th>Affected eye</th>
<th>Bacteriology</th>
<th>Complication</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dogs</td>
<td>1</td>
<td>German short-haired pointer</td>
<td>7-year-old male</td>
<td>OS</td>
<td>Pseudomonas aeruginosa</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>Japanese Spaniel</td>
<td>2-year-old female</td>
<td>OD</td>
<td>Pseudomonas aeruginosa</td>
<td>Extension of the keratomalacia</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Britain Spaniel</td>
<td>9-year-old male</td>
<td>OS</td>
<td>No growth</td>
<td>None</td>
</tr>
<tr>
<td>Cats</td>
<td>1</td>
<td>DSH</td>
<td>4-year-old neutered female</td>
<td>OS</td>
<td>Not Performed</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>DSH</td>
<td>8-year-old neutered female</td>
<td>OS</td>
<td>Not Performed</td>
<td>None</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>Persian</td>
<td>7-year-old neutered male</td>
<td>OD</td>
<td>Not Performed</td>
<td>None</td>
</tr>
</tbody>
</table>

RESULTS

Bacterial culture revealed infection with *Pseudomonas aeruginosa* in Dog 1 and Dog 2, sensitive to the antibiotics prescribed. No bacteria were isolated from the sample from Dog 3.

The follow-up examination performed at 1 week revealed prominent superficial and deep peripheral corneal neovascularization extending to the margins of the graft. Fluorescein staining was positive overlying the graft. Dog 2 presented with a painful eye; keratomalacia had extended to the previously uninvolved cornea as well as the BP graft, eventually leading to corneal perforation (Fig. 5). This animal underwent a successful bridge conjunctival graft after removal of the infected BP graft. Cat 3 developed moderate blepharospasm and serous ocular discharge with noticeable discomfort postoperatively; the other patients did not show signs of ocular discomfort.

At 2 weeks, corneal vascularization was still present, but the fluorescein staining was negative with the exception of Cat 3 that demonstrated a small epithelial defect over the graft. Ocular discomfort had resolved in all animals.

At 4 weeks, the corneal vascularization was regressing. The BP graft was opalescent in all patients (Figs 6 and 7) except for Dog 2 that had undergone the conjunctival graft placement and Cat 3 in which the BP graft remained opaque. Suture material was removed when still present.
OCT was performed on Dog 1 and Cat 3; epithelial healing was complete in both Dog 1 (Fig. 8) and Cat 3. The general shape of the cornea was normal in Dog 1, but Cat 3 demonstrated stromal ectasia and associated corneal deformation (Fig. 9). The transition between the graft and the recipient stroma was clearly visible; the underlying cornea appeared as a lamellar structure, whereas the BP graft appeared as a homogeneous, nonlamellar structure on which the epithelium migrated.

When the epithelial barrier was restored (as evidenced by slit-lamp examination and negative fluorescein staining), topical dexamethasone phosphate (Sterdex®; Théa, Clermont-Ferrand, France) was prescribed for each dog and topical indomethacin (Indocoll®; Chauvin, Montpellier, France) for each cat, twice daily over 2 weeks so as to minimize residual corneal vessels.

Two months after the surgery, the corneas of Dog 1 and Dog 3 were translucent, and only a focal central scar was visible; the scar was less visible in retroillumination (Fig. 10) than in transillumination (Fig. 11). A paracentral corneal scar was present in the three cats, and residual corneal vascularization was more prominent than in the dogs, despite the medical treatment (Fig. 12). OCT was performed on Dog 1 (Fig. 13) and Cat 3 (Fig 14). The epithelium appeared normal, and the cornea was no longer deformed in Cat 3. However, the corneal stroma was much thinner in the graft region in Cat 3. The transition between the graft and the stroma also remained visible with OCT.

No recurrence of the corneal sequestrum was observed in the cats up to 6 months postoperatively. All patients demonstrated vision in the affected eye, except Dog 2 where corneal opacification persisted following removal of the conjunctival graft.

**DISCUSSION**

Over the past decades, there has been growing interest in regenerative medicine, which aimed at repairing, replacing, and regenerating damaged tissues. BP grafts have been extensively used in human patients in different pathologic conditions including arterial or venous dissection, tracheal rupture, cardiac defects such as tetralogy of Fallot or atrial or ventricular septal defect, diaphragmatic resection, and abdominal wall defects. In the field of ophthalmology, BP patch transplantation has been described for the management of neurotrophic corneal ulcers, corneal perforation, corneo-scleral reconstruction after tumor resection, orbital floor reconstruction, and to cover glaucoma drainage devices or hydroxyapatite orbital implants.

In veterinary ophthalmology, various surgical techniques have been described for the management of deep melting corneal ulcers or corneal sequestra including conjunctival grafts, corneal-kera-toplasty, penetrating keratoplasty, amniotic membrane transplantation, equine renal capsule graft, porcine SIS graft. While these techniques are associated with a high success rate, degree of restoration of corneal transparency and thus quality of vision is variable. Optimal reconstruction would maintain a clear visual axis to preserve vision.
The use of SIS graft has been described by Vanore et al.\textsuperscript{22} in seven eyes (two cats and five dogs) presenting with deep melting corneal ulcers. No severe complications occurred, and vision was preserved in all the cases. Featherstone et al.\textsuperscript{20} described the use of SIS in 10 cases of feline corneal disease (four cases of deep stromal ulcers, one case of melting ulcerative keratitis, and five cases of corneal sequestra). One case of deep stromal ulcer required a complementary conjunctival graft because of early positive Seidel test. The melting ulcerative keratitis required enucleation secondary to extensive keratomalacia. The five cases of corneal sequestra were successfully treated by SIS graft. In a recent retrospective study of one hundred and six eyes treated with SIS graft,\textsuperscript{23} Goulle showed that vision was preserved in all eyes at 3 months postsurgery; in 69.8\% of the cases, the cornea was either transparent or scarring minimal and focal, while in 30.2\% of the cases, a more extensive scar was observed. Minor complications occurred in 8.5\% of the cases, including partial integration of the SIS, and in

Figure 8. Dog 1: Optical coherence tomography (OCT) aspect of the cornea 1 month postsurgery. The epithelial barrier is intact. The global shape of the cornea is normal.

Figure 9. Cat 3: Optical coherence tomography (OCT) aspect of the cornea 1 month postsurgery. The epithelium covers the graft. A corneal facet is present and affects the shape of the cornea. An important corneal edema is also visible.

Figure 10. Dog 1: retroillumination aspect of the eye 2 months postsurgery. The scar is translucent.

Figure 11. Dog 1: same picture in transillumination. The scar is more visible.

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22.6% faint or mild corneal pigmentation, without impairment of vision. In cases followed over a period longer than 3 months, progression of pigmentation impaired vision in five dogs.

Amniotic membrane transplantation has been described by Barachetti et al. for the treatment of feline corneal sequestra. In their study, corneal transparency was restored in five of seven treated eyes, with no recurrence of the disease 9 months after the surgery. Barros et al. reported the successful use of amniotic membrane in the management of severe bullous keratomalacia in a Yorkshire dog. Amniotic membrane transplantation has also been used in the management of a large corneal epithelial cyst in a dog, in the reconstruction of the cornea after the excision of dermoids and ocular tumors in dogs or ankyloblepharon in cats, and for ocular surface reconstruction in horses.

A review of reported success rates of different procedures in cases of deep melting corneal ulcers and corneal sequestra is summarized in Table 2.

Barros et al. have described the use of equine pericardium in the surgical treatment of experimentally created superficial keratectomy in twelve dogs. The study included a histologic analysis of the grafted cornea, at days 7, 15, 30, and 100 after implantation. The equine pericardial graft was well tolerated in all cases, despite a mild blepharospasm until day 15, and a chemosis for 1 week in all animals. An inflammatory cellular infiltrate was seen histologically during all the experiment. At day 100, histologic examination revealed fibrosis and mild inflammatory infiltrate. The epithelialization began at day 7 and was complete at day 30. The same team used equine pericardium in the surgical management of a corneal perforation and of a corneo-scleral reconstruction after the removal of a limbal melanoma in two dogs. The equine pericardium...
was well tolerated and allowed an accurate healing of affected tissues.

In our study, the patches were provided by Tutogen. Tutopatch is a xenograft stem from bovine pericardium. It is composed of collagen and elastin only. The patches are viro-inactivated and sterilized meaning they are cleaned of cells, bacteria, virus, DNA, and fungus. The rough side of the patch should be in contact with the corneal stroma to facilitate host cell migration and neovascularization. As for the SIS and amnion grafts, there is a specific orientation recommended by the manufacturer. The rough side of the Tutopatch promotes integration of the mesh and remodeling by the endogenous reparative tissue of the patient. The smooth side of the Tutopatch does not promote such adhesions.

The results of our clinical study are similar to those obtained with SIS graft or amniotic membrane transplantation. A successful outcome was obtained with BP graft in five of six eyes. In the solitary failure, we suspect that the BP had become infected by the organism responsible for the keratomalacia. Furthermore, the frequency of topical instillations of antibiotics on the infected corneas we implemented may have been insufficient for the medical management of the infection.

The biocompatibility of SIS with the cornea has been studied in rabbits. Greca et al. have compared the biocompatibility of SIS and BP used as grafts in the inferior cava veins of dogs; both biomaterials seem to behave similarly during the healing process. Barros et al. have studied the biocompatibility of equine pericardium with dogs cornea. Our results show that the healing phases of the cornea after BP graft were clinically similar to the SIS graft and equine pericardium graft, initially associated with marked vascularization of the peripheral cornea. This phase lasted between 1 and 2 weeks. The second phase was assimilation of the graft and re-epithelialization of the graft surface. The third phase of vascular regression and stromal remodeling toward transparency started approximately 3–4 weeks after the surgery. In the cats treated for corneal sequestra, corneal neovascularization persisted compared with dogs treated for deep melting corneal ulcers.

Anterior segment OCT imaging is widely used in human ophthalmology to evaluate, refine, and manage corneal transplantation patients. It allows a precise assessment of the interaction between the graft (whatever its type) and the recipient cornea. In our study, OCT examination demonstrated that the graft was assimilated by the cornea. Four weeks after the surgery, the epithelium was continuous over the graft. The interface between the exogenous and the endogenous collagen remained obvious 2 months after the surgery. Healing

| Table 2. Literature review of the results obtained with different surgical techniques for the treatment of melting corneal ulcers and corneal sequestra in dogs and in cats |
|-----------------------------------------------|------------------|------------------|
| Deep melting ulcers                          | Corneal sequestra |
| Number of cases | Success rate (%) | Reference | Number of cases | Success rate | Reference |
| Corneo-conjunctival transposition | 17 | 100% | Andrew et al. |
| Small intestinal submucosa graft             | 34 | 100% with no recurrence | Goulle |
| 42 dogs                                      | 100% with 4 recurrences | Goulle |
| 7 Cats                                       | 100% | Featherstone et al. |
| 5 dogs                                       | 100% | Featherstone and Sansom |
| 2 cats                                       | 100% | Featherstone and Sansom |
| Conjunctival graft                           | 5 | 100% | Featherstone et al. |
| 1 dog (360° conjunctival graft)              | 100% | Featherstone and Sansom |
| 1 dog (360° conjunctival graft)              | 100% | Featherstone and Sansom |
| 10 dogs (pedicle grafts and bridge grafts)   | 100% | Featherstone and Sansom |
| 14 dogs (pedicle graft)                      | 100% | Featherstone and Sansom |
| Amniotic membrane transposition              | 7 | 71% with no recurrence | Barachetti |
| 1 dog                                        | 100% | Pena Jimenez et al. |
| 3 horses                                     | 100% with no recurrence | Barachetti |
| Lamellar keratoplasty                        | 6 (allograft and heterograft) | 10% | Townsend et al. |
| Keratectomy alone                            | 1 | 0% | Townsend et al. |
|                                                | 44 | 100% with 1 recurrence | Townsend et al. |

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occurred without alteration in the global shape of the cornea both in the dog and in the cat. However, the stromal thickness was reduced in the region of the graft in the cat. While recurrence of sequestra was not observed, the duration of follow-up precludes definitive conclusions in this regard.

Secondary calcification has been described as a long-term complication after BP grafts in the human literature, and a longer follow-up period is required to assess this potential complication in our patients. The small number of cases included in our study does not allow us to make any definitive conclusion, but keratectomy associated with BP graft has provided effective treatment for corneal sequestra with resultant visual eyes. Further investigations are required to assess more accurately the efficacy of BP graft for the treatment of infected corneal ulcers.

REFERENCES


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