HUMAN AMNIOTIC MEMBRANE TRANSPLANTATION IN THE TREATMENT OF FELINE CORNEAL SEQUESTRUM: PRELIMINARY RESULTS

Lia ION¹, Iuliana IONASCU¹, Cárol GARCÍA de JOZ², Irene CERRADA², Alin BIRTOIU¹, Eduardo HUGUET²

¹University of Agronomic Sciences and Veterinary Medicine of Bucharest, Faculty of Veterinary Medicine, 105 Splaiul Independentei, 050097, Bucharest, Romania, Phone: +4021.318.04.69, Fax: +4021-318.04.98, Email: lyayon@yahoo.com, driulianaionascu@yahoo.com
²Oftalmovet, Veterinary Ophthalmology Practice, 32 Carrer de les Illes Canàries 46023, Valencia, Spain, Phone: +34 963 62 56 64, Email: oftalmomvet2@gmail.com

Corresponding author email: lyayon@yahoo.com

Abstract

Amniotic membrane transplantation is used in both human and veterinary ophthalmology for ocular surface reconstruction after certain ocular diseases in order to regain corneal transparency and to improve visual outcome. Feline corneal sequestrum is a disease unique to the cat, characterized by the presence of an area of corneal necrosis, brown coloured, sometimes accompanied by vascularization and edema, usually located in the center of the cornea. Depending on the stage of the disease, treatment can be medical or surgical. This pilot study aims to evaluate the clinical outcome after lamellar keratectomy and amniotic membrane transplantation in cases of feline corneal sequestrum. The study was conducted in the Ophthalmology Department of the Faculty of Veterinary Medicine in Bucharest and in Oftalmovet Private Practice in Valencia for a period of two years. During this time, six cats with corneal sequestrum underwent superficial keratectomy associated with human amniotic membrane transplantation fixed with OcuSeal Liquid Ocular Bandage™ (Beaver Visitec). In all the cases, a third eyelid flap was used to protect the graft. Good corneal clarity and improved vision was obtained in all the cases. Amniotic membrane transplantation after lamellar keratectomy was an optimal choice for the treatment of corneal sequestrum in cats. There was no recurrence of the disease in our follow-up period (3-15 months).

Key words: amniotic membrane, cat, cornea, corneal adhesive, sequestrum.

INTRODUCTION

Amniotic membrane transplantation (AMT) was first used in human ophthalmology in 1940 in the treatment of ocular burns (Rama et al., 2001; von Versen-Hoynek et al., 2004). After this, in 1990, Kim and Tseng wrote about their success in using human amniotic membrane for repairing various corneal defects (Kim et al., 2009; Sonia June Hill, 2008). In veterinary ophthalmology, Barros et al. (1998) were the first to report the use of equine amniotic membrane to repair corneal perforations in the dog (Storms et al., 2012).

Amniotic membrane (AM) is the innermost layer of the fetal membrane, consisting of an epithelium, a basement membrane and a stroma (Niknejad et al., 2008; Sonia June Hill, 2008). It contains various growth factors, hormones and cytokines (Cristina Peris and Menezo, 2004) that offer its different properties. Several studies have shown that AM has anti-inflammatory, antifibrotic and antiangiogenic effects (Tseng SC., 2001; Wang et al., 2001; Plummer, 2009), promotes migration of the epithelial cells, prevents corneal epithelium apoptosis and inhibits protease activity (Sangwan et al., 2007; Niknejad et al., 2008; Storms et al., 2012). Also, AM has antibacterial and antiviral activity (Dua et al., 2004; Fernandes et al., 2005; Plummer, 2009).

AMT can be recommended as a surgical therapy in different ocular diseases, like bullous keratopathy (Sangwan et al., 2007; Plummer, 2009), deep stromal ulcers and descemetoceles (Duchesne et al., 2001; Solomon et al., 2002), melting ulcers (Lassaline et al., 2005), corneal burns (Meller
et al., 2000; Sangwan et al., 2007), symblepharon (Barros et al., 2005; Barachetti et al., 2010). In human ophthalmology, AMT was also used in cases of limbal stem cell deficiency, amniotic membrane promoting cell differentiation (Grueterich et al., 2003; Barachetti et al., 2010). AM is able to cover the ocular surface like a graft after removal of some types of corneal or corneolimbal tumors (Barros et al., 2005; Ollivier et al., 2006) or after dermoid resection in dogs (Kalpravidh et al., 2009).

Corneal sequestrum is a disease that affects primarily the cat, but isolated cases have also been reported in dogs (Bouhanna et al., 2008) and in horses (McLellan et al., 2000). Brachycephalic cats are more commonly affected (Andrew et al., 2001; Barachetti et al., 2010), the lesion being usually localized in the central or paracentral cornea (Featherstone et al., 2004). The corneal stroma becomes necrotized and the lesion can extend in depth and in size (Laguna et al., 2014), leading to corneal perforation (Featherstone et al., 2004).

Predisposing factors include tear film abnormalities, lagophthalmos, ocular trauma, chronic corneal irritation (entropion, ectopic cilia, distichiasis) or the presence of feline herpesvirus-1 (Featherstone et al., 2004; Cullen et al., 2005; Grahn et al., 2005; Williams et al., 2009). In the first stage of the disease, when the necrosis is only superficial, the sequestrum can slough off just with medical therapy, represented usually by hyaluronic acid containing ophthalmic gels (Featherstone et al., 2004; Maggs, 2013). In cases where sequestrum is accompanied by areas of ulceration, antibiotic collyres are added. When the sequestrum is affecting the deeper corneal layers and there are associated ocular signs like epiphora, blepharospasm, vascularization, edema, surgery is recommended to remove the affected area (Featherstone et al., 2004; Maggs, 2013; Dulaurent et al., 2014). Most commonly, a superficial keratectomy is performed, followed by corneal grafting, which could be done by using conjunctival grafts or various biomaterials (Featherstone et al., 2001; Barachetti et al., 2010; Laguna et al., 2014).

AMT can be used for ocular surface reconstruction after removal of corneal sequestrum and can be fixed in place either by using sutures, usually 8-0/10-0 Vicryl (Vicryl® or Ethicon) or by using tissue adhesives (Barros et al., 2005; Barachetti et al., 2010; Lerit et al., 2012). OcuSeal® is a special liquid ocular bandage, a new type of tissue adhesive, made of a synthetic hydrogel. It has a high water content and is well tolerated by the ocular structures (Wathier et al., 2006; Ó hÉineacháin, 2011). In people, this tissue adhesive can be used to close the corneal incision after cataract surgery, after sclerotomies or pterygium surgery (Kim et al., 2006; Singh et al., 2010).

The present study aims to evaluate the visual outcome and the degree of corneal clarity after superficial keratectomy and sutureless human amniotic membrane transplantation in the therapy of feline corneal sequestrum.

MATERIALS AND METHODS

This study was conducted in the Ophthalmology Department of the Faculty of Veterinary Medicine in Bucharest and in Oftalmovet Private Practice in Valencia between December 2013 and December 2015. During this period, six cats with corneal sequestrum were treated using human AMT. Data collected for each case included breed, age, gender, clinical history, concurrent ocular diseases, eye affected, size and depth of the corneal necrosis. Initial ocular assessment was done in all the cases and included visual testing, examination with a light source and a magnifying loupe, Schirmer tear test, fluorescein stain, tonometry (TonoVet iCare, Finland), indirect ophthalmoscopy (PanOptic, Welch Allyn, NY, USA) and photodocumentation (Nikon D80 with Medical Nikkor 200 mm macro objective; Nikon D3200 with AF-s DX 18-55 mm objective).

Amniotic membranes used for transplantation in the present study were already prepared in a specialized laboratory. They were all obtained from cesarean births and kept in optimal conditions until use. All the cats went to general anaesthesia and the affected eye was aseptically prepared for ocular surgery with 2% povidone iodine solution. After the eye was fixed in place with stay sutures using 6-0 monofilament nonabsorbable suture material (Ethilon® Nylon Suture), a lamellar keratectomy was performed using a corneal knife to remove the corneal sequestra.
The keratectomy site was 1 mm larger than the corneal defect and the incision started from the external quadrant to the center of the cornea. The incision was deep enough as to remove all the brown pigment from the corneal stroma. After that, the remaining corneal defect was covered completely by a human amniotic membrane graft. The AM was placed in a single layer, with its stromal side facing the corneal stroma. The graft was then fixed in place using the liquid ocular bandage (OcuSeal®, Beaver-Visitec International, Waltham, MA). The corneal adhesive was prepared by mixing together its two components, the diluent and the powder, and shaking the container for 3 seconds. After that, the obtained gel was applied on the surface of the cornea with the special brush tip applicator within 10 seconds. If this time was not respected, the liquid became solid and couldn’t be used anymore. After polymerization on the surface of the cornea, the liquid bandage was transformed in a transparent, protective gel that fixed the AM graft. The excess of the liquid bandage was removed from the margins using a corneal scissors. A third eyelid flap was then used to protect the corneal stroma in two cats and 2/3 of the third eyelid flap was kept in place just for one week.

RESULTS AND DISCUSSIONS

The six cats affected by corneal sequestrum were represented by four Persian and two Domestic Shorthair cats. There were two neutered males, three spayed females and one intact female. The sequestrum was present in the right eye (OD) of three cats, left eye (OS) in two cats and affected both eyes (OU) in one cat. The Persian cat with bilateral corneal sequestrum underwent superficial keratectomy and AMT for the OS, while the OD sequestrum was managed with topical medication. The mean age of the affected animals was 5.8 years, with a range between 2 and 10 years old. Concurrent ocular disease included tear film deficiency in three cats, lower eyelid entropion in one cat and history of corneal ulceration in one cat. One of the Persian cats had a history of allergic dermatitis treated with oral prednisolone. The sequestrum affected 1/3 of the corneal stroma in three cats, 1/2 of the corneal stroma in two cats and 2/3 of the corneal stroma in two cats. Associated ocular signs were epiphora, blepharospasm, superficial vascularization and edema surrounding the corneal lesion. Table 1 summarizes the pretreatment ocular findings and visual outcome in the six patients.

Table 1. Signalment, pretreatment characteristics and clinical outcome in six cats with corneal sequestrum

<table>
<thead>
<tr>
<th>Case</th>
<th>Breed</th>
<th>Age</th>
<th>Gender</th>
<th>Affected eye</th>
<th>Concurrent ocular disease</th>
<th>Visual outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>DSH</td>
<td>7yr</td>
<td>M(C)</td>
<td>OS</td>
<td>Tear film deficiency</td>
<td>Good, superficial corneal vascularization</td>
</tr>
<tr>
<td>2</td>
<td>Persian</td>
<td>4yr</td>
<td>F(S)</td>
<td>OD</td>
<td>Tear film deficiency</td>
<td>Good</td>
</tr>
<tr>
<td>3</td>
<td>Persian</td>
<td>5yr</td>
<td>M(C)</td>
<td>OD</td>
<td>Chronic corneal ulceration</td>
<td>Central corneal ulceration</td>
</tr>
<tr>
<td>4</td>
<td>DSH</td>
<td>2yr</td>
<td>F</td>
<td>OD</td>
<td>Lower eyelid entropion</td>
<td>Good</td>
</tr>
<tr>
<td>5</td>
<td>Persian</td>
<td>10y</td>
<td>F(S)</td>
<td>AO</td>
<td>Tear film deficiency</td>
<td>Good, small central corneal scar</td>
</tr>
<tr>
<td>6</td>
<td>Persian</td>
<td>7yr</td>
<td>F(S)</td>
<td>OS</td>
<td>None</td>
<td>Good</td>
</tr>
</tbody>
</table>

DSH: Domestic Shorthair cat; yr: years; M(C): neutered male; F: intact female; F(C): spayed female; OD: right eye; OS: left eye

Tarsorrhaphy was maintained for 21 days in four of the affected cats. In one cat (Case 1, Table 1) sutures from the third eyelid flap were removed after six weeks (Figure 1e), while for one cat (Case 6, Table 1) these were kept in place just for one week.
After surgery, all cats received oral antibiotics, either amoxicillin/clavulanic acid (Synulox®, Pfizer) 12.5 mg/kg q 8 h or doxycycline (Ronaxan®, Merial) 10mg/kg q 24 h for 10 days. Also, they were all administered oral nonsteroidal antiinflammatories (robenacoxib, Onsior®, Novartis) 1 mg/kg q 24 h for 3 days. Tobramycin (Tobrex®, Alcon) and Diclofenac (Voltaren®, Novartis), three times daily, were used topically in two of the cats postoperatively.

For the other four cats topical therapy was started after tarsorrhaphy suture removal and consisted in antibiotic and artificial tears eye drops, antiinflammatory eye drops being added 10-14 days later in order to reduce the granulation tissue proliferation. As a long term therapy, aminoacids and hyaluronic acid ophthalmic gel (HyCare®, Bausch&Lomb) was recommended in almost all the cases, especially in the cats that had tear film deficiencies.

Case two (Figure 2), a 4 years old female Persian cat developed a small ulceration in the center of the cornea one month after AMT. Local treatment was instituted, consisting in antibiotic eye drops, anticollagenolytics (N-acetylcysteine) and artificial tears gel, given six times daily, with improvement of the clinical signs after one week. Bilateral corneal sequestrum was present in Case 5 (Table 1), this Persian cat having also the history of allergic dermatitis. The sequestrum in OD was treated topically with antibiotic eye drops and HyCare®, six times daily and the area of necrosis has sloughed off after one month. In OS superficial keratectomy and AMT were performed (Figure 3a), sutures from tarsorrhaphy being removed after 21 days, when the cornea had a small area of opacity in the supero-extern quadrant and superficial vascularization (Figure 3b). After 5 days, Dexamethasone containing eye drops were started three times daily, with good clinical outcome at the future revisits. Nine weeks after surgery, a thin corneal scar was present and also two small areas that stained with fluorescein. At this moment, antibiotic eye drops and artificial tears were recommended. The last check, 4 months after surgery, revealed a transparent cornea, with good visual outcome (Figure 3f).
Figure 2. Case 2: a - Clinical aspect at initial examination: small central area of necrosis, surrounded by edema and peripheral vascularization; b - Performing superficial keratectomy with the corneal knife; c - After AMT. The graft is larger than the corneal defect; d - AM graft fixed with the liquid ocular bandage; e - Clinical aspect one week after removal of the tarsorrhaphy sutures. Central corneal opacity and superficial blood vessels; f - Same picture as in “e”, lateral aspect. Superficial, thin scar.

Figure 3. Case 5: a - Superficial keratectomy. 1/3 deep corneal sequestrum, accompanied by superficial vascularization; b - 21 days after surgery, small area of opacity; c - 5 days after tarsorrhaphy suture removal. Dexamethasone eye drops were started; d - 45 days after surgery, good clinical outcome; e - Nine weeks after keratectomy and AMT. Thin corneal scar; f - 4 months after surgery. Transparent cornea, negative fluorescein test.
Case 6, a 7 years old female Persian cat had a three weeks history of epiphora and brown pigmentation of the skin around the left eye. The sequestrum was affecting one third of the corneal stroma and was staining the fluorescein dye at the periphery (Figure 4a). In this cat, after keratectomy and AMT, the third eyelid flap was removed in 7 days, when a good adhesion of the AM graft was noticed (Figure 4c). Topical treatment with antibiotic, nonsteroidal antiinflammatorys and artificial tears was instituted, with a good clinical outcome. Three weeks after surgery, a small central area of epithelial denudation appeared (Figure 4e), but a complete remission was seen after 7 days. The last recheck was after five months, when the cornea was completely transparent (Figure 4f).

**CONCLUSIONS**

Feline corneal sequestrum affects primarily brachycephalic cats and is characterized by an area of corneal necrosis, brown pigmented, usually located in the central or paracentral cornea. Recommended treatment can be medical or surgical, depending on the depth of the lesion.

In the present study, a superficial keratectomy was performed in order to remove the corneal sequestrum, followed by a human amniotic membrane transplantation and a third eyelid flap.

Amniotic membrane promotes reepithelialization, inhibits neovascularization and fibrotic tissue formation by suppressing the fibroblasts and has antiinflammatory effects. Being avascular, there is rare graft rejection after AMT. Because the AM is transparent, its use as a graft for corneal surface reconstruction supports cosmetic repair. At the moment, AMT is probably the best option in the surgical management of feline corneal sequestrum.

In our study, sutureless AMT was used after lamellar keratectomy. AM graft without sutures has some advantages, like shorter surgical time and reduction of suture-induced inflammation. For fixing the AM on the corneal surface, a liquid ocular bandage was used. This is a transparent adhesive that forms a protective barrier for the cornea. The disadvantage is that
it has to be prepared and used very quickly after reconstitution otherwise it becomes solid and cannot be used anymore.

The six cats that we treated with AMT after lamellar keratectomy for the therapy of feline corneal sequestrum had a good visual outcome and corneal transparency was noted in the follow-up period. There was also no recurrence in neither of the cats.

REFERENCES


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