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Equine Amniotic Membrane Transplantation in Corneal Perforation Resulting from Melting Ulcer in a Dog

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Abstract : A 4-year-old castrated male Shih-Tzu was presented to the Chonbuk National University Animal Medical Center with a history of melting ulcer in the right eye (OD). Upon ophthalmologic examination, severe keratomalacia of approximately 70% of the entire surface area with a full thickness corneal defect was found in OD. In addition, iris and fibrin clots were observed on the center of the corneal defect. The menace response and dazzle reflex were normal in OS. As the size and damage of corneal perforation was so severe, amniotic membrane (AM) transplantation was considered to repair the cornea instead of direct suture technique, flap methods or corneal transplantation. Equine AM was sutured to the limbus to cover the entire cornea in a single interrupted pattern using 9-0 nylon suture material. On day 79, mild scarring and pigmentation, with almost no vasculature, remained. The menace response and dazzle reflex were normal of OD. Although pigmentation and scarring remained on the cornea, equine AM transplantation can be useful for reconstruction of severe corneal perforation with keratomalacia.

Key words **:** full thickness corneal defect, melting ulcer, amniotic membrane, keratomalacia, dog.

Introduction

Severe corneal damage is a challenging condition to treat surgically, however corneal perforation is a more frustrating situation for veterinary clinician (2). Corneal perforation occurs when Descemet's membrane is breached and a full-thickness lesion is created, resulting in aqueous humor loss and possibly iris prolapse. This can lead to anterior synechiae, glaucoma, cataract, endophthalmitis, or loss of vision (5,14). Therefore, prompt and effective treatment is extremely important.

Corneal perforations have been treated with conjunctival flaps and grafts, corneoscleral or corneoconjunctival graft, cyanoacrylate adhesives, small intestinal submucosa (SIS), pericardium or peritoneum. However, in many instances, it is difficult to apply the techniques because of technical or supply problems (8-11,15). In addition, it is hard to be applied the criteria including tectonic support, transparency, and minimal scarring of the corneal treatment (13). In the case of amnion, it is proper for the treatment goals of providing tectonic support, transparency, and minimal scarring (13). Therefore, amniotic membrane (AM) has been widely used in human ophthalmology because of the advantage of proper strength, anti-angiogenic, and anti-inflammatory factor.

In veterinary ophthalmology, AM has been used for reconstructing the ocular surface, including conjunctiva, sclera, and cornea, especially in dogs. Barros *et al*. (1998) used equine AM to repair corneal perforation in experimental cases (2). They also reported AM transplantation for ocular surface repair in three clinical canine cases (3). Canine and bovine AM transplantation was reported for corneal reconstruction in dermoid excision and created corneal ulcers, respectively (6). Recently, Choi *et al*. (2010) reported equine AM transplantation for corneal epithelial inclusion cyst excision in a dog (4). However, using equine AM to severe corneal perforation by melting ulcer has seldom been reported. Therefore the purpose of this paper is to describe the equine AM transplantation in a canine case of severe corneal perforations by melting ulcer.

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Case

A 4-year-old castrated male Shih-Tzu was referred to the Chonbuk National University Animal Medical Center due to ocular discharge and epiphora on the right eye (OD). One week prior to the visit, the patient was diagnosed on canine distemper virus infection and presented diarrhea, hyperthermia, ocular and nasal discharge and panting. No special treatment was given for the poor eye condition; therefore, both eyes had presented severe ocular discharge, epiphora, and blepharospasm.

During physical examination, body temperature and respiratory rates were within the normal ranges. Serum biochemical findings were in normal range however, hematologic analysis showed mild lymphopenia. Upon ophthalmologic examination, severe keratomalacia of approximately 70% of the entire sur-

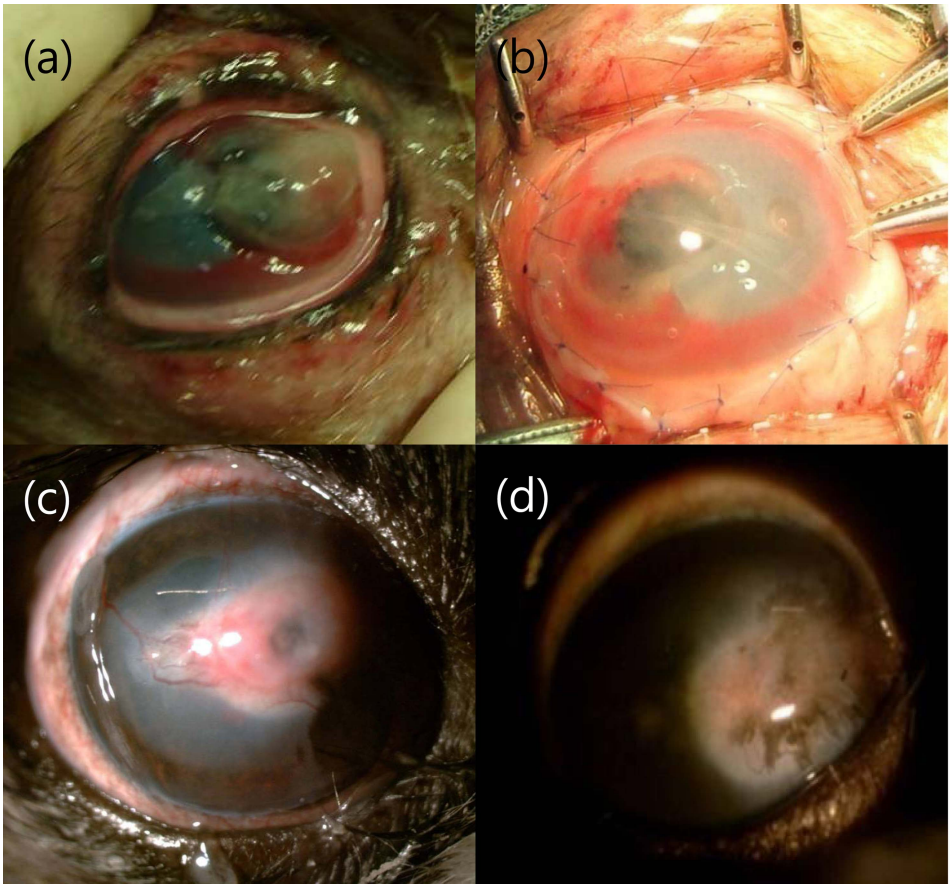

Fig 1. (a) Large corneal melting ulcer with corneal perforation. (b) Transplanted equine amniotic membrane above the damaged cornea. (c) On day 36 postoperatively, neovascularization and scarring could be observed. (d) On day 79 postoperatively, complete healing of the cornea was shown. Note the reduced scarring and mild pigmentation.

face area with a full thickness corneal defect was found in OD. In addition, iris and fibrin clots were also observed on the center of the corneal defect (Fig 1a). Owing to the severe corneal damage, menace response and dazzle reflex were absent. Additionally, Schirmer tear test and tonometry were not conducted. Menace response, dazzle reflex and pupillary light reflex (PLR) were intact in OS. An ocular swap was performed on the site of perforation for antibiotics susceptibility test of OD.

As the size of corneal perforation and stromal damage were so wide and severe, equine AM transplantation was determined for reconstruction of the cornea rather than lamellar corneal or corneoscleral transposition, corneal allografts. Prior to the surgery, the patient was treated with topical eye drops, including ofloxacin (Ocuflox®, Samil, Korea), 1% atropine (Ocutropine®, Samil, Korea), systemic medication with cephalexine (Falexin®, Dongwha, Korea) and carprofen (Rimadyl®, Pfizer, Sandwich, UK) based on antibiotics susceptibility test. Surgery was performed under general anesthesia with 1.5% to 2% isoflurane (Forane®, Choongwae, Korea) after induction with 1% propofol (Provine®, Claris, India) and premedication with atropine sulfate (Jeil, Korea). The prolapsed iris and fibrin clots were removed and debridement of the cornea was performed. Then, the equine AM was transplanted to the damaged cornea. Equine AM was sutured to the limbus to cover the entire cornea in a single interrupted pattern using 9-0 nylon suture material (Fig 1b). The AM preparation was performed according to the established procedure of the authors' lab (4). Prior to transplantation, equine AM was obtained from a fetal membrane following a live birth. The amnion was separated from the chorion and rinsed with phosphate buffered saline (PBS). The amnion was then stored with penicillin, streptomycin, and neomycin at −80ºC. Sections were thawed as needed and rehydrated in sterile saline solution.

The autoserum q12hr was added to the preoperative medications: ofloxacin (Ocuflox®, Samil, Korea) q6hr, 1% atropine (Ocutropine®. Samil, Korea) q12hr, systemic medication with cephalexine (Falexin®, Dongwha, Korea) and carprofen (Rimadyl®, Pfizer, Sandwich, UK) q12hr. There was no aqueous humor leakage, and the anterior chamber and the shape of globe were maintained well, with mild protrusion of the AM for the first few days after surgery. 1 day after AM transplantation, the cornea showed inflammation with corneal neovascularization and mucous ocular discharge. One week after AM transplantation, corneal vessels were extended to the AM graft and the AM became absorbed to the next week.

1 month after AM transplantation, extended granulation tissue and vascularization were observed (Fig 1c). Two months later, the scarring decreased, and pigmentation appeared in the margin of the scar. At day 79, mild scarring and pigmentation, without any vasculature, were only shown (Fig 1d). The menace response and dazzle reflex were normal also IOP was in normal range as 13 mmHg in OD.

Discussion

Corneal perforation accompanied by iris prolapse is a devastating condition caused by deep corneal ulcer or trauma. The reported case was corneal perforation due to corneal melting ulcer. The patient presented a severe melting ulcer priory infected by canine distemper virus. The exact cause of corneal ulcer in this case was unknown, although the previous canine distemper virus infection was suspected to have affected the development of ulceration. It is well known that keratoconjunctivitis sicca (KCS) is one of the clinical signs of canine distemper infection (10,12). In canine patients suffering from KCS, approximately 17% were affected with corneal ulcer (12). Although a low percentage, melting ulcer in this case could be suggested that canine distemper induced KCS developed corneal ulcer and secondary bacterial infection caused melting ulcer.

The goal of treating corneal perforation is preventing the leakage of aqueous humor from the anterior chamber and controlling the inflammation (5). Full thickness defects in the cornea have been treated using conjunctival autografts, cyanoacrylate adhesives, lamellar corneal or corneoscleral transposition, corneal allografts or biomaterials including SIS, pericardium or peritoneum. Conjunctival grafts and cyanoacrylate adhesives may be used for partial thickness defects, or for small, focal, corneal penetrations, but for larger full thickness defects, lamellar keratoplasty, penetrating keratoplasty, or corneoscleral transposition have been used more favorable (8-11,15). Corneoscleral or corneoconjunctival graft methods have the advantage of using the autogenous tissue. However, corneoscleral transposition has limitations. The size of the lesion that can be reconstructed may be restricted by the amount of tissue that is available, or the amount that may be used has a possibility of scarring at the donor site (9,15). Biomaterials including small intestinal submucosa (SIS), pericardium, peritoneum and amnion have been used to reconstruct the damaged cornea in veterinary ophthalmology. However, the difficulty with a majority of the materials, including porcine small intestinal submucosa SIS, pericardium, and peritoneum are the source location (8-11). In this case, the corneal defect was too large for direct suture, whereas penetrating keratoplasty was difficult to find a donor and also risk of rejection. Autogenous corneal or cornel sclera grafts were also not an option because the adjacent cornea was too edematous to tight firmly the sutures. According to the previous literatures and author's experience, we decided to transplant equine AM to the corneal defect in this case. AM has the advantage of proper strength, anti-angiogenic, and anti-inflammatory factors. AM could be used as a graft or patch. For grafts, AM is trimmed a little larger than the size of the defect, anchored in place with the basement membrane side facing up. Therefore, it functions as a basement membrane on which the corneal epithelium can grow. AM is spread over the whole cornea and anchored at the limbus, when using as patch. It then functions as a bandage and also acts as a barrier to protect the corneal from inflammatory cells and proteins in the tear film (1). In the present case AM was used as patch and sutured to the limbus because the adjacent tissue could not offer proper strength for suturing.

In veterinary ophthalmology, AM has been used in reconstruction of ocular surface, especially for cornea (3,4,6-8,11, 14). The repair of partial defected cornea with amniotic membrane was reported by some authors in small animals. Barros *et al*. (2005) reported AM transplantation in three different cases, bullous keratomalacia, symblepharon and resection of fibrous histocytoma, using canine AM. All of the three cases presented good outcome (3). Equine AM transplantation to feline sequestrum cases after lamellar keratectomy was reported with high success rate in selected cases (1). Kalpravidh *et al*. (2009) described AM transplantation after superficial keratectomy for dermoid and Choi *et al*. (2010) reported AM transplantation after corneal epithelial inclusion cyst excision, respectively (4,6). Otherwise, AM transplantation for full thickness defect of the cornea was only reported by Barros *et al*. (1998) (2). Corneal perforation was created in canine experimental models and equine AM was grafted to the defected cornea. Although this report was similar corneal perforation case that need full thickness repair, the most difference was the adjacent tissue. This case has severely edematous cornea of the entire stroma that could not provide proper tension even for suture whereas the cornea nearby experimental corneal perforation had adequate tension for suturing. This situation made AM grafting hardly, therefore AM patching method was selected to repair the cornea. The outcome of repair the full thickness defect can suggest that AM as patching acts not only as a protection barrier but also basement membrane. The cornea was also well reconstructed after AM transplantation; however, mild scar and pigmentation remained, because of a severe melting and perforation in the presented case. This case presented good reconstruction of the damaged cornea, without any additional procedure, including third eyelid flap or conjunctival flap.

This case reported herein was a successful clinical application of equine AM transplantation to treat corneal perforation resulting from melting ulcer in dog. Because this is a single case, further studies with a larger number of cases would be required to support the use of equine AM in corneal perforation in dogs.

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녹는 궤양에 기인한 각막 천공에서 각막 재건을 위한 말양막이식술 증례

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요 약 : 4년령 중성화한 수컷 시츄견이 우안에 녹는 궤양이라는 기왕력으로 전북동물의료센터에 내원했다. 안과 검사 결과 우안에 각막 전체의 70%에 해당하는 각막 연화를 동반한 각막 천공이 관찰되었다. 또한 홍채 탈출과 섬유소 응괴가 각막 천공 중앙부에서 관찰되었다. 좌안의 위협 반사와 대광 반사는 정상이었다. 각막 결손 부위가 너무 컸기 때문에 각결막 판을 이용한 방법이나 각막 직접 봉합을 배제하였으며 말양막이식술을 시행하기로 결정했다. 말양막은 각막 윤부에 전체 각막을 다 덮을 수 있도록 9-0 나일론 봉합사를 이용하여 봉합을 실시했다. 양막이식 후 79일 째 육아조직과 색소침착이 다소 남아있었으나 우안의 위협 반사와 눈부심 반사는 모두 정상이었다. 비록 색소 침착과 육아조직이 각막에 남아있기는 했지만 개에서 말양막이식술은 녹는 궤양으로 기인한 각막 천공에서 시행해 볼 수 있는 유용한 치료법으로 생각된다.

주요어 : 전층 각막 결손, 녹는 궤양, 양막, 각막 연화증, 개