



Clinical Reports

Deep anterior lamellar keratoplasty (DALK) as a treatment of a deep stromal ulcer in a dog: case report

Ceratoplastia lamelar anterior profunda (DALK) como tratamento de úlcera estromal profunda em cão: relato de caso

Gabriel Godoi de Moraes^{1*} , Bruno Marostica Mambelli² , Fernando Bezerra da Silva Sobrinho¹ 

¹ Universidade Estadual Paulista (UNESP), Faculdade de Ciências Agrárias e Veterinárias (FCAV), Jaboticabal, SP, Brasil

² Clínica Veterinária BMvet, Ribeirão Preto, SP, Brasil

ARTICLE INFO

Article history

Received 28 April 2020

Accepted 29 June 2020

Keywords:

Keratopathy

Transplantation

Ulceration

ABSTRACT

Characterized by the rupture of the corneal epithelium, corneal ulcers threaten ocular integrity, with multifactorial development, such as decreased protection and excessive abrasion applied to the corneal surface. Keratoplasty restores the corneal anatomy, removing the inflamed or infected tissues, in order to promote visual acuity. The technique of deep anterior lamellar keratoplasty (DALK) selectively removes the diseased layers of the cornea, keeping the Descemet membrane and endothelium healthy, reducing the risk of rejection, aimed at tissue recovery. In the present report, a canine, from the Shih Tzu breed, a female, that has not been castrated, 2 years and 6 months old, was treated, she had a history of ocular discomfort and mucous secretion in both eyes. In the ophthalmic examination, keratoconjunctivitis sicca was found in the left eye and ectopic cilia was found in both eyes. With the aid of a fluorescein test, the presence of a deep stromal ulcer affecting the right eye was confirmed. The patient underwent ectopic cilia removal using the wedge resection technique and an allogeneic corneal transplantation using DALK, with only the diseased epithelium and stroma being replaced, reducing the risk of rejection and failures related to surgery. 90 days after the procedure, there was no reaction to the graft, there was complete epithelial repair and maintenance of visual function with slight corneal opacity.

RESUMO

Caracterizada pela ruptura do epitélio corneano, as úlceras de córnea ameaçam a integridade ocular, tendo desenvolvimento multifatorial, como a diminuição da proteção e o excesso de abrasão aplicado na superfície da córnea. A ceratoplastia restaura a anatomia corneana, removendo tecidos inflamados ou infectados, com objetivo de promover acuidade visual. A técnica de ceratoplastia lamelar anterior profunda (DALK - deep anterior lamelar keratoplasty) remove seletivamente as camadas doentes da córnea, mantendo a membrana de Descemet e endotélio saudáveis, reduzindo o risco de rejeição, visando recuperação tecidual. No presente relato, um canino, raça Shih Tzu, fêmea não castrada, 2 anos e 6 meses de idade, foi atendido com histórico de desconforto ocular e secreção mucosa em ambos os olhos. No exame oftálmico constatou-se ceratoconjuntivite seca no olho esquerdo e cílios ectópicos em ambos os olhos. Com auxílio do teste de fluoresceína, confirmou-se a presença de úlcera estromal profunda acometendo o olho direito. O paciente foi submetido a remoção dos cílios ectópicos pela técnica de secção em cunha e ao transplante de córnea alógena utilizando a DALK, sendo substituído apenas o epitélio e estroma doentes, reduzindo o risco de rejeição e falhas relacionadas a cirurgia. Após 90 dias do procedimento, constatou-se ausência de reação ao enxerto, completa reparação epitelial e manutenção da função visual com discreta opacidade corneana.

Palavras-chave:

Ceratopatias

Transplante

Ulceração

* Corresponding author: gabriel.godoi.rp@gmail.com

INTRODUCTION

Among the ophthalmic diseases, corneal ulcers have a high casuistry in veterinary medicine, affecting a large number of animals, occurring more frequently in brachycephalic dogs due to their facial shape, with shallow orbits and prominent eyes, making the eyeball more exposed, the English Bulldog, Pekingese, Pug and Shih Tzu breeds among the most predisposed (AMORIM et al., 2018; RAMANI et al., 2012).

Characterized by the rupture of the corneal epithelium, ulcerations represent a threat to the integrity of the eyeball, consequently leading to impaired vision. Depending on the number of layers reached, ulcers are classified as superficial when they are restricted to the epithelial layer or as being deep when they reach the stroma. Stromal ulcerations are divided into superficial, anterior, medium or deep depending on the depth of the injury caused to the cornea (MERLINI et al., 2014; RAMANI et al., 2012).

Multiple factors are related to their development, highlighting traumatic causes, ectopic cilia, deficiency of tear production, chemical lesions, eyelid defects, bacterial infections, allergic, metabolic, endocrine and neurotrophic diseases (MERLINI et al., 2014; VYGANTAS; WHITLEY, 2003). Among the main clinical signs of the pathology the following stand out eye discomfort and pain, photophobia, blepharospasm, presence of periorbital secretion, epiphora and loss of corneal transparency (MERLINI et al., 2014; RAMANI et al., 2012; VYGANTAS; WHITLEY, 2003).

The treatment of the lesion aims to preserve the eyeball and maintain visual function, to assist healing, inhibit the formation of an edema and prevent the multiplication of microorganisms that can aggravate the condition (OLLIVIER, 2003; VYGANTAS; WHITLEY, 2003). During the evolution of ophthalmology, several techniques have been developed and, among them, corneal transplantation or keratoplasty has been gaining space in veterinary medicine, consisting of the replacement of the diseased cornea, partially or in whole, with another one coming from a donor in order to restore the corneal anatomy, removing inflamed or infected tissues, aimed at reestablishing visual function (KIM et al., 2016; SANO et al., 2008; TAN et al., 2012).

Deep anterior lamellar keratoplasty (DALK) is applied in order to selectively replace the diseased layers of the cornea, retaining those that are healthy, reducing the risk of rejection of the transplanted tissue. This way, DALK removes varying amounts of the corneal stroma and epithelium, maintaining the Descemet membrane and endothelium, thereby allowing the affected cornea to support its recovery after transplantation (COSCARRELLI; NEVES; BOTEON, 2012; PRAZERES et al., 2016; SANO et al., 2008; TAN et al., 2012).

Although there are several surgical treatments to repair corneal defects and perforations, such as a conjunctival

pedicle graft, corneal scleral transposition and the use of biological membranes from, for example, the intestinal submucosa, amniotic membrane, pericardium and renal capsule, these techniques are unable to restore corneal transparency (BARBOSA et al., 2013; BRAGA et al., 2004; KIM et al., 2016). Thus, a corneal transplantation using DALK is an alternative, since the correct application of the technique allows for the maintenance of the shape and transparency of the transplanted allogeneic cornea (BRAGA et al., 2004; KIM et al., 2016).

Despite being one of the main methods used for visual rehabilitation in human medicine, the use of keratoplasty in veterinary medicine is still scarce due to the low availability of donated tissue, in addition to the need for technical training for the use of a surgical microscope (TAN et al., 2012).

The present work aims to report on the use of DALK to repair deep stromal ulcers in a dog.

CASE REPORT

This report describes the use of DALK for the treatment of a deep stromal ulcer diagnosed in a Shih Tzu canine, a female, 2 years and 6 months old, weighing 5 kg. The animal had a history of ocular discomfort and the presence of a large amount of mucus-like secretion in both eyes for 2 days.

The ophthalmic examination was performed using a rebound tonometer (Tonovet), slit lamp and a light source. In the right eye, there was a pupillary, consensual and ambulation reflex present, a positive threat test, ectopic cilia and periorbital mucous secretion. Upon examination of the right cornea, vascularity, diffuse opacity and a positive fluorescein test for the continuity of a solution in the presence of a cobalt blue light were observed. Inspection of the anterior chamber, iris and pupil was limited due to the opacity implanted in the right cornea. In the left eye, there was a pupillary, consensual and ambulation reflex present, a positive threat test, a 13 mm Schirmer, IOP of 15 mmHg, ectopic cilia and periorbital mucous secretion. Examination of the left cornea showed dorsal vascularization, paracentral pigmentation and a negative fluorescein test, demonstrating the absence of ulceration in the presence of cobalt blue light (Figure 1).

Figure 1. A - Presence of ectopic cilia, conjunctival hyperemia, chemosis, a deep stromal lesion stained with fluorescein, corneal vascularization and a diffuse edema in the right eye. B - Presence of vascularization, pigmentation, an edema, chemosis, hyperemia and ectopic cilia in the left eye.



At the end of the evaluation, the patient was diagnosed with an ulcer and ectopic cilia in the right eye. The ulcerated area affected the corneal stroma and epithelium, being classified as being a deep stromal ulcer. In the left eye, quantitative keratoconjunctivitis sicca and ectopic cilia were found. Furthermore, the presence of conjunctival hyperemia, chemosis, superficial vascularization and the diffuse edema in the right eye are indicative that the lesion has progressed in a chronic way. Complementary tests were also performed, such as blood count and serum biochemical: urea, creatinine, ALT and albumin, the values being within the physiological parameters of the species.

The treatment adopted for the patient was to perform a corneal transplant, together with the surgical removal of the ectopic lashes using the wedge resection technique, using a surgical microscope with a 36-fold magnification at the focal point. Treatment for quantitative keratoconjunctivitis sicca was also performed.

Drug treatment, for ophthalmic use, was instituted with 0.03% tacrolimus eye drops (Tacrolimus 0.03% eye drops DrogaVet®, 1 drop/eye every 12 hours, until further recommendations), moxifloxacin hydrochloride (Vigamox®, 1 drop/eye every 4 hours, for 7 days) for the local antibiotic therapy, eye drop based on disodium EDTA 0.35% (eye drop EDTA disodium 0.35% DrogaVet®, 1 drop/eye every 4 hours, until further recommendations) were used, aiming to reduce the proliferation of microorganisms and the metalloproteinases activity, and lubricating eye drops based on hyaluronic acid (Ocucan®, 1 drop/eye every 8 hours, until new recommendations) were used, for the lubrication and restoration of the tear film. For oral use, amoxicillin with clavulanate (Agemoxi® CL, 15 mg/kg, every 12 hours, for 5 days), prednisone (Meticorten®, 0.5 mg/kg, every 12 hours, for 7 days), dipyrone (Dipyrone Biovet®, 1 drops/kg every 8 hours, until further

recommendations), and omeprazole (Gaviz® 10 mg, 0.8 mg/kg, every 24 hours, for 5 days) was prescribed.

The patient was subjected to a solid food fast of 6 hours and a liquid fast of 2 hours prior to surgery. Subcutaneous Acepromazine (Acepran®, 0.03 mg/kg) was applied as the pre-anesthetic medication. In a continuous act, anesthetic induction with propofol (Dipivan®, 4 mg/kg) was performed intravenously, with the subsequent orotracheal intubation using a 6.0 tube connected to a semi-open anesthetic device. Isoflurane (minimum alveolar concentration of 1.3%) was used for anesthetic maintenance in a calibrated vaporizer. A wide trichotomy was performed in the periorbital region, placing the patient in the supine position, raising the head using a support. Antisepsis of the operative field was done using 5% povidone iodine drops (5% PVPI eye drops Centro Paulista Laboratório®) in the eye and 10% povidone iodine (Antiseptic Povidine®) on the skin. The field cloths were placed by the surgeon, who then applied, in sequence, an incisional adhesive surgical field (Dermabond®).

The removal of the canine cornea from the donor was performed 3 days before the surgical procedure. After collection, the cornea was cleaned with gentamicin sulfate solution (Gentamicin sulfate Allergan® ophthalmic solution), and then kept refrigerated, with an average temperature of 6°C, in a sterile universal disposable collector (Evil Care®), suspended in tobramycin (5ml Tobrasynine Syntec® eye drops) until the moment of transplantation.

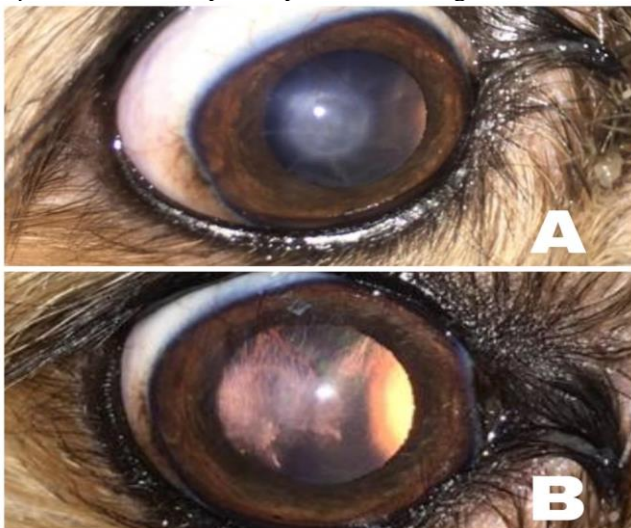
With a 36-fold magnifying surgical microscope, the DALK was performed using a 4 mm punch, causing a circular lesion in the recipient cornea, subsequently with the aid of a crescent blade and an iris spatula, the removal was performed, removing the epithelial portions and sick stromal. In sequence, lesion and remodeling were carried out, ranging from the epithelium to the corneal Descemet membrane to be transplanted with the same 4 mm punch. The donor cornea was transplanted over the lesion in the recipient cornea. Suturing was applied in a separate simple pattern, using 9-0 nylon, with the first stitches fixed at the cardinal points, with the needle being passed from the donor cornea towards the recipient.

An evaluation performed 5 days after the procedure demonstrated the presence of corneal edema and vascularization, with no blepharospasm (Figure 2). The stitches were removed at 30 days, with the aid of a 36-fold magnifying surgical microscope, using general anesthesia. 45 days after the surgery, a reduction in opacity with a reduction in corneal edema was observed, with the presence of phantom vessels also being noticed due to the resolution of the inflammatory process achieved through the surgical procedure. At 90 days after the surgery, there was no graft rejection, with complete epithelial healing as well as the maintenance of visual function (Figure 3).

Figure 2. - Appearance at 5 days after the deep anterior lamellar keratoplasty (DALK) procedure, one can notice corneal edema and superficial vascularization towards the transplanted cornea.



Figure 3. A - Endothelial edema observed 45 days after the surgical procedure. B - Appearance after 90 days of the surgical procedure, notice the absence of any graft rejection with complete epithelial healing.



The patient remained comfortable, with no discomfort or eye pain, at all times during the postoperative evaluation. The graft integrity assessment was accompanied by a biomicroscopy and funduscopy with a slit lamp and a tonometry with a rebound tonometer. The maintenance of visual function was verified through a threat test, glare test and obstacle test.

DISCUSSION

The case reported is in agreement with the authors Amorim et al. (2018) and Ramani et al. (2012), when mentioning that corneal ulcers mostly affect brachycephalic and young dogs due to the shape of their faces, with shallow orbits and prominent eyes, making the eyeball more exposed, thus encompassing the patient in question, as it belongs to the Shih Tzu breed, being 2 years and 6 months old when it was diagnosed with a deep stromal ulcer. In addition, the patient had ectopic lashes in both eyes and quantitative keratoconjunctivitis

sicca in the left eye, which according to Merlini et al. (2014) and Vygantas; Whitley (2003) are the most predisposing factors for the occurrence of ulcers in animals, causing corneal injury due to friction and poor lubrication of the eyeball.

A point to be highlighted is that antibiotic therapy is necessary in the treatment of corneal ulcers, aiming to eradicate bacterial infection, preventing further tissue destruction and preventing the latter from becoming more serious (OLLIVIER, 2003; VYGANTAS; WHITLEY, 2003).

The choice for deep anterior lamellar keratoplasty as opposed to penetrating keratoplasty is in accordance with Tan et al. (2012), since the technique is considered to be safer, with better results and less possibilities of postoperative complications. In addition, the absence of impairment of the Descemet membrane makes it unnecessary to use a penetrating keratoplasty, however, in case of accidental corneal perforation it must be used, so the veterinary surgeon must be prepared for the application of both techniques (SANO et al., 2008; TAN et al., 2012).

The main advantage associated with the DALK procedure is the fact that the technique maintains the tissues that are healthy, replacing only the diseased epithelium and stroma, reducing the chances of possible rejections and failures related to the transplant (COSCARELLI; NEVES; BOTEON, 2012; PRAZERES et al., 2016; SANO et al., 2008; TAN et al., 2012). This affirmation was demonstrated in the study by Kim et al. (2016) in three eyes of three male beagles submitted to the DALK procedure, in which there was no graft rejection after 150 days of follow-up of the population, and only the formation of a small cloudiness was found around the transplanted margin between the donor and recipient corneas. In the present case, the application of the DALK procedure also did not cause any graft rejection, maintaining the patient's visual function with only slight corneal opacity.

Conjunctival pedicle grafts are indicated for protection and support of ulcerations, however, they are unable to promote total transparency of the cornea, and its use is often related to vision loss at the site of its fixation, thus corneal transplant using the DALK procedure is an alternative, since it provides better corneal transparency (BRAGA et al., 2004; KIM et al., 2016). In the present report, the use of the DALK procedure provided the maintenance of the patient's visual function with only slight corneal opacity observed at 90 days after the surgery.

The application of the DALK procedure to the reported patient provided good visual results, complete epithelial healing, no rejection of the transplanted cornea, maintenance of visual function and the absence of discomfort and eye pain, in agreement with the results observed by the authors Kim et al. (2016), Sano et al. (2008) and Tan et al. (2012) in their studies. However,

the difficulty in acquiring the corneal tissue to be transplanted often makes it impossible to use the technique for the treatment of more severe ulcers (TAN et al., 2012).

CONCLUSIONS

The protocols used to perform the DALK procedure provided no reaction to the graft, with complete epithelial repair and the maintenance of visual function with only slight corneal opacity. In addition, the patient remained comfortable, with no discomfort or eye pain, at all times during the postoperative evaluation.

REFERENCES

- AMORIM T. M. et al. Impacto do grau de uveíte em diferentes tipos de úlceras de córneas em cães submetidos ao enxerto pediculado de conjuntiva bulbar – 34 casos. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. v. 70. n. 4. p. 1233-1239, 2018.
- BARBOSA A. et al. Lamellar keratoplasty in dogs using equine amniotic membrane. *Clinical and morphological study*. *Brazilian Journal of Veterinary Research and Animal Science*. v. 50. n. 3. p. 211-219, 2013.
- BRAGA F. V. A. et al. Lamellar keratoplasty produced by free corneal autograft and conjunctival pedicle fixed with cyanoacrylate adhesive in dogs. *Ciência Rural*. v. 34. n. 4. p. 1119-1126, 2004.
- COSCARELLI S. A.; NEVES R. C.; BOTEON J. E. Ceratectomia lamelar anterior profunda usando a técnica “big-bubble” em pacientes com ceratocone. *Arquivos Brasileiros de Oftalmologia*. v. 75. n. 1. p. 20-23, 2012.
- KIM S. et al. Deep anterior lamellar keratoplasty of dog eyes using the big-bubble technique. *Journal of Veterinary Science*. v. 17. n. 3. p. 347-352, 2016.
- MERLINI N. B. et al. Uso de plasma rico em plaquetas em úlceras de córnea em cães. *Arquivo Brasileiro de Medicina Veterinária e Zootecnia*. v. 66. n. 6. p. 1742-1750, 2014.
- OLLIVIER F. J. Bacterial corneal diseases in dogs and cats. *Clinical Techniques In Small Animal Practice*. v. 18. n. 3. p. 193-198, 2003.
- PRAZERES T. M. B. et al. Visual outcomes after deep anterior lamellar keratoplasty using donor corneas without removal of Descemet membrane and endothelium. *Arquivos Brasileiros de Oftalmologia*. v. 79. n. 6. p. 366-368, 2016.
- RAMANI C. et al. Incidence of corneal ulcer in dogs – A retrospective study. *Veterinary & Animal Science*. v. 8. n. 5. p. 250-252, 2012.
- SANO F. T. et al. Trends in the indications for penetrating keratoplasty. *Arquivos Brasileiros de Oftalmologia*. v. 71. n. 3. p. 400-404, 2008.
- TAN D. T. H. et al. Corneal transplantation. *The Lancet*. v. 379. n. 9827. p. 1749-1761, 2012.
- VYGANTAS K. R.; WHITLEY R. D. Management of deep corneal ulcers. *Compendium on Continuing Education for the Practicing Veterinarian*. v. 25. n. 3. p. 196-205, 2003.