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## Limbal autograft transplantation in a dog with alkali-induced ulceration

### Transplante de limbo autógeno em cão com úlcera por álcali

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#### - NOTE -

#### ABSTRACT

*A case of a 3-month-old female mongrel dog with a history of apathy and previous contact with an alkaline (sodium hydroxide) is reported. The dog was reluctant to open the right eye. Ophthalmic examination revealed blepharospasm, photophobia, epiphora, discrete chemosis, conjunctival hyperemia, and diffuse corneal edema involving the limbus. The fluorescein test was positive and the result of the Schirmer tear test was 32mm min<sup>-1</sup>. On the basis of these findings the diagnosis was alkali-induced ulceration and limbal autograft transplantation was performed. Corneal vascularization was observed by the third postoperative day, with intensification in vessel number and caliber on subsequent days and small areas of corneal transparency. The present results show that limbal autograft transplantation is a feasible procedure for the therapeutic management of alkali-induced corneal ulcers.*

**Key words:** *cornea, ulcerative keratitis, sodium hydroxide, transplant.*

#### RESUMO

*Descreve-se um caso de um animal da espécie canina, fêmea, sem raça definida, de três meses de idade, com histórico de apatia, contato prévio com produto alcalino (hidróxido de sódio) e relutância em abrir o olho direito. Ao exame oftálmico, foram observados blefarospasmo, fotofobia, epífora, quemesa discreta, hiperemia conjuntival, e edema corneal difuso com comprometimento do limbo. Foram realizados o teste da fluoresceína positivo e o Teste Lacrimal de Schirmer 32mm min<sup>-1</sup>. Com base nos achados, firmou-se o diagnóstico de úlcera por álcali e realizou-se transplante autógeno do limbo. No pós-operatório, observou-se*

*vascularização corneal a partir do terceiro dia e sua intensificação, em número e calibre, nos dias subsequentes. Também foram observadas mínimas áreas de transparência corneal. Os resultados obtidos permitem admitir que o transplante autógeno de limbo é procedimento factível para o manejo da terapia de úlceras de córnea por álcali.*

**Palavras-chave:** *córnea, ceratite ulcerativa, soda caustica, transplante.*

Chemical burns, especially those caused by alkaline substances, or severe heat burns can damage the ocular surface with serious complications such as persistent epithelial defects, trophic ulcers, ischemic necrosis and, consequently, loss of corneal transparency and even ocular perforations (THOFT & FRIEND, 1977). These events particularly occur when, in addition to destruction of the corneal and conjunctival epithelia, there is important involvement of the limbus (SHAPIRO et al., 1981).

Various therapies for the repair of corneal damage have been proposed, including the use of blood serum (JORGE et al., 2003), substances rich in linoleic acid (CALADO et al., 2005), and substances that inhibit metalloproteinases such as extracts of *Pothomorphe umbellata* (BARROS et al., 2007) and experimental surgical procedures (BRUNELLI et al., 2006).

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If the entire limbus is affected, alterations such as migration of the conjunctival epithelium to the corneal surface (conjunctivalization), chronic inflammation, vascularization, calcification and ulceration, and corneal melting and perforation tend to occur (DUA & AZUARA-BLANCO, 2000). Limbal transplants have been suggested as possible alternatives (DUA & AZUARA-BLANCO, 1999; BRUNELLI et al., 2006).

The objective of this note was report the clinical case of a dog with alkali-induced ulceration which was submitted to limbal autograft transplantation.

A 3-month-old female mongrel dog was seen with a history of apathy and intermittent blepharospasm lasting for 6 hours. The owner reported that the animal had come in contact with a product containing sodium hydroxide (NaOH). No systemic alterations were observed upon physical examination. Slit lamp biomicroscopy<sup>a</sup> revealed blepharospasm, photophobia, epiphora, discrete chemosis, conjunctival hyperemia, diffuse corneal edema involving the limbus, and corneal surface irregularities in the affected right eye. The results of the Schirmer tear test<sup>b</sup> in the affected eye were above normal (STT=32mm min<sup>-1</sup>). Indirect binocular ophthalmoscopy<sup>c</sup> of the affected eye could not be performed because of the presence of diffuse corneal edema. The cornea was stained with fluorescein<sup>d</sup> throughout its extension (Figure 1A). No alterations were observed in the contralateral eye.

After abundant washing of the ocular surface with sterile saline at 8°C, the animal was submitted to inhaled general anesthesia and surgical treatment. The surgical field was prepared and protected routinely. Two lamellae of the healthy limbus measuring approximately 0.5cm were collected from the contralateral eye under the light of a surgical microscope. For this purpose, lamellar dissection was performed at the corneal periphery, extending up to 3mm into the conjunctiva, at 11 to 12 and 7 to 9 o'clock. The excised tissue was kept in sterile saline at room temperature immediately after excision (BRUNELLI et al., 2006). The limbal lamellae collected were then transplanted into the affected eye. Simple interrupted total non-penetrating 9-0 nylon monofilament sutures at a distance of 1mm were used for fixation. The donor and recipient eyes were treated with topical antibiotic (tobramycin) at regular intervals of 6 hours for 30 consecutive days. Atropine 1% was administered at regular intervals of 12 hours for 5 days.

Ophthalmic examination of the eye receiving the transplant revealed blepharospasm, photophobia, epiphora, discrete chemosis, conjunctival hyperemia,

diffuse corneal edema, and corneal surface irregularities on the first two days after surgery. Discrete epiphora and chemosis were observed in the contralateral eye. On the third day, corneal vascularization was noted close to the transplanted limbus (Figure 1B). There were signs of ocular discomfort accompanied by discrete blepharospasm and epiphora. In the donor eye, discrete chemosis was observed close to the site of graft excision.

On the subsequent days, especially on day 15, the fluorescein test was found to be negative and vessels were still present in large numbers, but no corneal transparency was observed. However, signs of ocular discomfort were no longer present. Discrete conjunctivalization was noted in the donor eye (Figure 1C). The use corticoids help in controlling conjunctivalization, but could decrease the process of tissue repair.

On postoperative day 21, vessels were still present in moderate numbers and small areas of corneal transparency were observed close to the transplant. On day 30 and 45, the cornea presented central vascularization and very discrete and poorly significant areas of transparency were observed close to the transplanted limbus (Figure 1D). No conjunctivalization was observed in the affected eye. At the end of the observation period, no complications were observed in the donor eye, except for a spot close to the area where the limbus was excised.

Signs of limbal alterations or deficiencies include photophobia, epiphora, blepharospasm, a red eye, and reduced visual acuity resulting from corneal edema (DUA & AZUARA-BLANCO, 1999), signs also observed in the present case.

Conjunctivalization in the injured area has been observed in animals clinically treated for alkali-induced ulceration (JORGE et al., 2003; CALADO et al., 2005). Conjunctivalization is the result of destruction of the limbus and, consequently, of its stem cells (DUA & AZUARA-BLANCO, 1999). BROWN et al. (1969) reported that limbal defects result in little or no neogenesis of corneal vessels, a fact probably contributing to the occurrence of conjunctivalization.

Limbal stem cells are responsible for tissue repair and regeneration (AKPED & FOSTER, 1999) and are essential for the maintenance of corneal surface integrity, promoting renewal of the cornea under physiological conditions and reepithelization in abrasive lesions (HAAMANN et al., 1998). Therefore, limbal transplants have advantages when compared to other therapeutic alternatives. BRUNELLI et al. (2006) reported the onset of corneal vascularization by the third day in animals with alkali-induced ulceration



Figure 1 - Photographic image of the right eye of a 3-month-old female mongrel dog with alkali-induced ulceration. A) Note the diffuse edema and positive fluorescein test. B) Note the diffuse edema with areas of vascularization close to the graft (arrows) 3 days after surgery. C) Note the discrete conjunctivalization at the site where the limbus was excised (arrows) and preserved corneal transparency 15 days after surgery. D) Note the conjunctivalization at the graft site and the small areas with discrete reduction in corneal edema (arrows) 45 days after surgery.

submitted to limbal transplantation. The same was observed in the present case.

In the present case, regeneration of the corneal and limbal epithelium was observed in the eye receiving the transplant 15 days after transplantation and limbal transplantation resulted in rapid and satisfactory vascularization. The same events have been reported by TAN et al. (1996). No corneal conjunctivalization, which would result in the permanent loss of corneal transparency, was observed. After 45 days, corticosteroids and sodium chloride 4% were used to reduce corneal edema, without showing decreased edema. Corneal edema persisted in the affected eye despite transplantation due to the penetrating action of alkali and the presence of stromal injury and possible cellular damage to the corneal endothelium.

In this case, little can be done to result in improved transparency, because the endothelial cells do not exhibit regeneration process. To get transparency on with corneal endothelial damage,

penetrating corneal transplant is needed. However, this technique was not yet routinely in veterinary medicine.

BRUNELLI et al. (2006) observed ocular, predominantly mucopurulent, secretion in the donor eye of 91.67% of dogs 3 days after limbal stem cell autograft transplantation. At 7 days, only 20% of the animals presented discrete mucoid secretion, whereas no ocular secretion was observed in the remaining 80%. Ocular secretion was no longer observed in all animals by day 14. These alterations were more discrete when compared to the present case, possibly because of the smaller donor area.

In conclusion, limbal autograft transplantation was found to be useful for the treatment of alkali-induced corneal ulceration since it contributes to the maintenance of the ocular surface without conjunctivalization. However, was not satisfactory in this case to promote transparency due to corneal endothelial damage. In addition, the healing events observed in the donor eye are poorly aggressive and do not impair visual function. The present case report

is the first to describe a limbal autograft to correct clinical corneal ulceration in a dog.

## SOURCES OF MANUFACTURES

- a - Schirmer tear test - Ophthalmos, Ribeirão Preto, SP, Brazil.
- b - Slit lamp - Kowa Company Ltd.
- c - Indirect binocular ophthalmoscope (Omega 180) - Heine Optotechnik.
- d - Fluorescein – Ophthalmos, Ribeirão Preto, SP, Brazil.

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