Treatment of bullous keratopathy with corneal collagen cross-linking in two dogs


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CASE REPORT

Treatment of bullous keratopathy with corneal collagen cross-linking in two dogs

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Abstract

Objective Corneal collagen cross-linking with riboflavin and UV-A (CXL) decreases corneal oedema and increases visual acuity in human patients with bullous keratopathy. Presumed mechanisms are an increase in collagen packing density and a reduction in stromal swelling pressure. We present two cases in which CXL was used to treat bullous keratopathy in dogs.

Procedures Four eyes of two dogs with painful bullous keratopathy-induced corneal erosions that were resistant to prior therapy were treated with CXL. Both corneas of the second patient were dehydrated to $400 \mu m$ corneal thickness using topical 70% glycerol solution immediately prior to CXL. Follow-up included slit-lamp examination, fluorescein staining and photographic documentation in both cases and high-resolution ultrasound examination in the second patient.

Results All four eyes were comfortable and fluorescein negative at 1-week post-CXL and remained so for the rest of the follow-up period (17.5 months for case 1 and 6 months for case 2). The owner of the first patient reported a less oedematous cornea and improvement in vision that lasted for 6 months. Despite a reported lack of improvement in vision in the second patient, corneal thickness initially decreased, but was back at baseline thickness at the 4-month recheck.

Conclusions Similar to humans, CXL might become a useful treatment option for bullous keratopathy-induced therapy-resistant corneal erosions in dogs. Patient comfort was greatly improved, but corneal thickness decrease was not as long-lasting as reported for humans. The presently used protocols might need modification to fit the dog cornea.

Key Words: bullous keratopathy, cornea, corneal collagen cross-linking, dog, endothelial decompensation, oedema

INTRODUCTION

Corneal collagen cross-linking (CXL) is based on the exposure of the photosensitizer riboflavin (Vitamin B2) to UV-A light with a wavelength at the riboflavin absorption peak of 370 nm. This results in a free oxygen radical-driven photopolymerization process introducing additional covalent cross-links within collagen fibres and in the proteoglycan–glycosaminoglycan ground substance of the corneal stroma up to a depth of 300 $\mu m$.1,2 The result is an increase in the biomechanical and biochemical stability of the cornea, an increase in collagen packing density and a reduction in the swelling tendency of the glycosaminoglycan-rich hydrophilic ground substance of the cornea. Reactive oxygen species (ROS)-induced damage to cells and reduction in microorganisms in the irradiated area has been described as well.1–8

After CXL was introduced for the treatment of progressive keratoconus in humans,9 new indications for the clinical use of CXL emerged rapidly, including the treatment of
melting keratitis, Fuchs’ dystrophy and bullous kerato- 
apthy. Melting keratitis and bullous keratopathy are two 
interesting indications for the use of CXL in veterinary 
medicine, as these diseases are potentially blinding and 
can be difficult to control both medically and surgically. The 
effective use of CXL for the treatment of melting keratitis 
in dogs, cats and horses was recently reported.

Various congenital and acquired diseases including 
breed-related endothelial dystrophy, iris-to-cornea persist-
ten pupillary membranes, trauma (both surgical and non-
surgical), anterior uveitis/endotheliitis, glaucoma, toxic 
damage to the endothelium, age-related endothelial 
degeneration and melting keratitis can cause functional 
decompensation of the corneal endothelium and result in 
bullous keratopathy. Pain and discomfort are caused by 
the recurrent or nonhealing corneal erosions that occur 
with bullous keratopathy. The dense corneal oedema 
results in significant loss of vision and even blindness in 
severe cases. Topical application of hypertonic solutions 
and ointments can decrease the extent of epithelial 
keratolysis and bullae formation, but typically does not 
significantly decrease the extent of the stromal oedema and 
opacification. The frequent necessary application and local 
irritation can limit the usefulness of topical hypertonic saline 
treatment. More invasive treatments including ther-
keratectomy, amniocentesis and conjunctival flap (Gunderson 
flap) placement can be used successfully to treat the ep-
ithelial defects, but these treatments further decrease cor-
neal transparency. Partial coverage of the corneal surface 
with a thin conjunctival flap can function as a drainage 
sink for the oedematous fluid in the stroma. This has been 
observed to subjectively increase corneal transparency (P. 
Bedford, personal communication; I. Allgoewer, personal 
communication); however, the duration of this effect is 
unknown.

Corneal collagen cross-linking with riboflavin and 
UV-A (CXL) decreases corneal oedema and increases 
visual acuity in human patients with bullous keratopathy. 
Presumed mechanisms are an increase in collagen packing 
density and a reduction in the swelling tendency of the 
glycosaminoglycan-rich hydrophilic ground substance of 
the cornea. The objective of this case report was to evaluate 
the effectiveness of CXL for the treatment of bullous keratopathy in dogs.

METHODS

Four eyes of two dogs with a two-month history of persist-
ten corneal erosions as a result of bullous keratopathy 
were treated with CXL at the veterinary clinical teaching 
hospital of the Veterinary (Vetsuisse) Faculty of the Uni-
versity of Zurich, Switzerland.

Pretreatment and post-treatment examinations

Both patients underwent slit-lamp examination (SL 15; 
Kowa Optimed Europe Ltd., Sandhurst, UK), fluorescein 
staining (Fluorescein Sodium; Acrivet-Veterinary Division, 
Hennigsdorf, Germany), Schirmer tear testing (Schirmer 
Tear Test; Intervet/Schering-Plough Animal Health, 
Roseland, NJ, USA), tonometry (TonoVet; ICare, Vantaa, 
Finland), indirect ophthalmoscopy (Omega 500, HEINE 
Optotechnik, Herrsching, Germany; 2.2 PanRetinal and 
28D lenses, Volk Optical Inc., Mentor, OH, USA) and 
photographic documentation (Nikon D90 camera and 
Nikon AF Micro Nikkor 60 mm lens; Nikon AG, Egg, 
Switzerland) at the initial visit. Slit-lamp examination, 
fluorescein staining and photographic documentation were 
performed during all follow-up visits. Corneal thickness 
measurements were performed only on patient 2 during 
the baseline examination, during the CXL procedure and 
at each recheck appointment. The corneal thickness 
measurements before and after CXL and at the first two 
timepoints during the CXL procedure were obtained via 
high-resolution ultrasound (40 MHz probe, Eyecubed I-3 
System, version 4; Ellex, Innovative Imaging, Sacramento, 
CA, USA). Corneal thickness was measured from each of 
the B-scan images using the ultrasound unit’s internal cal-
pers. The measurements at the second through eighth 
timepoint during the CXL procedure were taken with an 
ultrasound pachymeter (PachPen handheld ultrasound 
pachymeter; Accutome, Inc., Malvern, PA, USA). The sec-
ond measurement during the CXL procedure was taken 
with both modalities with <40 μm difference between 
measurements. All pachymetry measurements were aver-
aged (2–4 measurements per timepoint). Total follow-up 
was 17.5 months for case 1 and 6 months for case 2.

Patients

Patient 1, a 13-year-old male neutered Shepherd mix, had 
been diagnosed with anterior uveitis in both eyes (OU) by 
the referring veterinarian 2 months prior to referral. A 
Rickettsia coronii infection was the presumed aetiology 
and treatment with systemic doxycycline (Supraycyclin, 
Grunenthal, 10 mg/kg orally twice daily for 3 weeks) and 
topical prednisolone acetate 1% (Pred Forte; Allergan, 
Inc., Irvine, CA, USA) and diclofenac 0.1% (Voltaren, 
Novartis International AG, Basel, Switzerland) was started 
at this time. Complete ophthalmic examination at the vet-
inary clinical teaching hospital of the Veterinary (Vets-
uisse) Faculty of the University of Zurich, Switzerland, 
revealed diffuse, noncystic corneal oedema, central epither-
elial erosions, mild inferotemporal corneal neovasculariza-
tion and blepharospasm. Menace responses, dazzle and 
pupillary light reflexes were present OU and no signs of 
an active uveitis were observed. An intraocular pressure 
(IOP) of 7 mmHg was measured in the left eye (OS) and 
8 mmHg in the right eye (OD). Schirmer tear test mea-
surements were within normal limits at all measured time-
points. A fundic examination was severely limited by the 
lack of corneal transparency and patient cooperation. Oral 
doxycycline was continued and topical neomycin/poly-
mixin/bacitracin ointment (Ophthalmymet, Vetoquinol)
was started three times daily OU. During the 2 months following this visit, the patient was repeatedly seen by an ECVO Diplomate in private practice (LWR), who confirmed the diagnosis of endothelial decompensation and absence of active uveitis. During this period, the patient was treated twice with cotton tip applicator and diamond burr (Algerbrush II; The Alger Company, Inc., Lago Vista, TX, USA) debridement and bandage contact lens placement (Acrivet Pat D2, Acrivet). Following these treatments, the patient was comfortable with the contact lenses in place, but discomfort returned due to the presence of persistent corneal erosions as soon as the contact lenses were removed. Treatment with topical broad spectrum antibiotics was continued throughout the treatment period.

The patient was again referred to the Veterinary (Vetsuisse) Faculty of the University of Zurich, Switzerland, for CXL treatment OU. Microcystic corneal oedema, corneal epithelial bullae and erosions, mild to moderate inferotemporal corneal neovascularization and pigmentation and multifocal depigmentation of the nictitating membrane were observed OU (Fig. 1a). CXL was performed OU under general anaesthesia as previously described. A bandage contact lens (Acrivet Pat D2, Acrivet) was placed OU immediately after CXL. Treatment was started with topical ofloxacin (FloxaL, Bausch & Lomb Swiss AG, Zug, Switzerland) eye drops three times daily OU and oral tramadol (Tramadol Mepha; Mepha Pharma AG, Basel, Switzerland) at 3–5 mg/kg up to three times daily as needed for pain. The patient was discharged from the clinic on the day of the procedure. Via telephone follow-up the next day, the owner reported conjunctival redness and intermittent blepharospasm, which resolved during exercise.

Both eyes were comfortable and both contact lenses were removed (LWR) 1 week after the CXL procedure. The corneas were both fluorescein negative.

Two weeks after the CXL procedure, both corneas were comfortable, subjectively less oedematous and fluorescein negative with a smooth epithelial surface. The corneal neovascularization and pigmentation and the depigmentation of the nictitating membrane were unchanged and interpreted as being consistent with pre-existent chronic superficial keratitis. Topical treatment was started with cyclosporine 0.2% (Optimmune, MSD Animal Health GmbH, Luzern, Switzerland) three times daily and neomycin/polymyxin/dexamethasone 0.1% (Maxitrol, Alcon, Fort Worth, TX, USA) four times daily OU. These medications were to be tapered to twice daily OU over 8 weeks. At recheck, 2 months later, both corneas remained comfortable and fluorescein negative with a smooth epithelial surface (Fig. 1b). The corneal and nictitating membrane pigmentation were unchanged and the

**Figure 1.** Clinical effect of Corneal collagen cross-linking (CXL) on bullous keratopathy (two patients). Bullous keratopathy as a result of endothelial decompensation was diagnosed in both patients. The appearance OD of case 1 immediately prior to CXL (a). The appearance OS was identical to OD. Note the dense microcystic corneal oedema and epithelial erosion. Fine pigmentation accompanied the neovascularization advancing towards the centre of the cornea (a). The appearance OD 2 (b) and 15 (c) months post-CXL. Both eyes were comfortable with a smooth, fluorescein-negative corneal surface and subjectively less intense corneal oedema. Minimal progression of the pigmentation was present. The appearance of case 2 immediately prior to CXL (d). Note the dense microcystic corneal oedema OU and the irregularity of the corneal surface (OS > OD). A central epithelial erosion was present OU. Radially oriented areas of pigmentation OD and neovascularization OS were present. One week post-CXL, both eyes were comfortable with a smooth, fluorescein-negative corneal surface and less intense corneal oedema (e).
corneal neovascularization had completely regressed OU. Treatment with cyclosporine 0.2% was continued twice daily and neomycin/polymyxin/dexamethasone 0.1% was tapered and discontinued over a 3 month period of time. The owner reported an improvement in visual performance at the 3- and 5-month postprocedure rechecks. At 6.5 months after the CXL procedure, the owner reported decreased corneal transparency and deterioration of visual performance, which were both difficult to confirm clinically. Topical treatment with 5% sodium chloride ointment (Muro 128, Bausch & Lomb) 3–4 times daily OU was added to the cyclosporine treatment. During the rest of the follow-up period up to a recheck at 17.5 months after the CXL procedure, no further changes were observed (Fig. 1c). Due to mild local irritation, application of the 5% sodium chloride ointment was tapered to twice daily OU.

Patient 2, a 14-year-old female castrated Jack Russell Terrier, presented with dense corneal oedema OS and mild to moderate corneal oedema OD. The corneal surface was irregular OU with faint multifocal fluorescein staining OS and a small epithelial erosion OD. Schirmer tear test measurements were within normal limits at all measured time-points. Corneal thickness was 0.97 mm OD and 1.28 mm OS as measured by high-resolution ultrasound. A complete examination of the anterior chamber was not possible due to the loss of corneal transparency. The iris seemed slightly hyperpigmented. However, no signs of an active uveitis were observed. The patient was diagnosed with corneal endothelial decompensation, which was presumed to be age-related or uveitis-induced based on the iris hyperpigmentation and low IOPs of 3 mmHg OS and 4 mmHg OD that were measured. Topical treatment was started with 5% sodium chloride ointment four times daily OU. Topical anti-inflammatory treatment was postponed due to the observed corneal surface abnormalities and a follow-up examination was recommended 2 weeks later.

The patient then presented 2 months later with a history of intermittent blepharospasm since the previous visit. No change was observed with the use of 5% sodium chloride ointment, and the episodes of blepharospasm were increase in length and frequency. Ophthalmic examination revealed dense microcystic corneal oedema, irregularity of the corneal surface, central epithelial erosions OU, radially oriented areas of pigmentation OD and neovascularization OS (Fig. 1d). Corneal thickness had increased to 1.2 mm OD and 1.43 mm OS.

Corneal collagen cross-linking was performed OU under general anaesthesia as previously described. Both corneas of the second patient were dehydrated to a corneal thickness of approximately 400 μm using topical 70% glycerol solution immediately prior to CXL according to a previously published protocol. This was carried out to increase the proportion of total stromal thickness reached by CXL, which has a penetration limit of ±300 μm. Treatment with 5% sodium chloride ointment three times daily was continued and topical neomycin/polymyxin/bacitracin ointment four times daily OU, oral tramadol 3–5 mg/kg up to three times daily as needed and robenacoxib (Onsior, Novartis) 1 mg/kg once daily with food for 3 days were started. Contact lenses were not placed and the patient was discharged from the clinic on the day of the procedure.

One week after the CXL procedure, both eyes were comfortable and fluorescein negative with a dense microcystic corneal oedema and smooth corneal surface

![Figure 2](https://example.com/figure2.png)

Figure 2. Corneal pachymetry data at various timepoints before, during and after Corneal collagen cross-linking (CXL) (patient 2). High-resolution ultrasound (HRUS) image of the central cornea and anterior lens capsule (white arrow) OS of case 2 taken 1 week after CXL (a). A demarcation line was visible between the anterior third and posterior two-thirds of the corneal stroma. The anterior stroma (asterisk) was hyperrechogenic compared to the posterior stroma. Digital callipers were used for corneal thickness measurements (yellow line). Corneal thickness of case 2 in μm plotted over time (b). An increase in corneal thickness between the first presentation and the presentation immediately prior to CXL had been observed. The decrease in corneal thickness measured during the CXL procedure was caused by the riboflavin–dextran 20% (decrease between first and second measurement) and glycerol 70% solutions (residual decrease). Four months after CXL, the corneal thickness had returned to the values measured immediately prior to CXL.
Corneal thickness was 1.06 mm OD and 1.2 mm OS. A demarcation line was visible on ultrasound between the anterior third and posterior two-thirds of the stroma (Fig. 2a). Only topical treatment with 5% sodium chloride ointment three times daily was continued. Apart from mild progression of pigmentation OD and neovascularization OS, the clinical situation at 1 month after the CXL procedure was identical to the corneal appearance 1 week postprocedure. Corneal thickness was 0.94 mm OD and 1.28 mm OS. Topical treatment with 5% sodium chloride ointment three times daily OU was continued and topical treatment with cyclosporine 0.2% and ketorolac 0.5% (Acular, Allergan) twice daily OU was initiated. During the remainder of the follow-up period at 6 months after the CXL procedure, no further changes in visual performance, patient comfort or clinical appearance of the corneas were observed. At the 4-month recheck, corneal thickness was 1.2 mm OD and 1.46 mm OS. However, topical treatment with 5% sodium chloride ointment was discontinued due to irritation. Topical treatment was continued with cyclosporine 0.2% twice daily and ketorolac 0.5% once daily.

RESULTS AND DISCUSSION

All four eyes included in this case report had persistent/recurrent corneal epithelial erosions present for at least 2 months prior to CXL treatment. These four eyes were comfortable and fluorescein negative 1 week following CXL treatment and remained so for the rest of the follow-up period: 17.5 months for case 1 and 6 months for case 2. No adverse effects of the CXL procedure, such as delayed epithelial healing, postprocedural pain or infection were observed. Pigmentation of the cornea was present prior to CXL in both patients and appeared to have progressed slightly 1 month after CXL in the second patient. Further progression of the corneal pigmentation was not observed in either patient while being treated with cyclosporine 0.2% twice daily OU.

The primary treatment goal, namely resolution of the corneal erosions and associated pain, was fulfilled, indicating that CXL may be a successful treatment alternative for dogs with bullous keratopathy. This result is consistent with the current literature, as various case series reported the effective use of CXL for the treatment of corneal oedema, endothelial decompensation and bullous keratopathy in humans.11,18–22

Corneal collagen cross-linking seems to have led to a subjective increase in corneal transparency in case 1 and to an objective, although not clinically significant, thinning of the cornea in case 2. The effect was temporary in both cases, which is consistent with the current clinical physician-based ophthalmic literature.11,21–23

Wollensak et al.24 described a significant decrease in the swelling of the anterior corneal stroma after CXL in order to fit both the dog cornea and to optimize the treatment of bullous keratopathy.

DISCLOSURE

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