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Corneal collagen cross-linking as treatment for infectious and noninfectious corneal melting in cats and dogs: results of a prospective, nonrandomized, controlled trial

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Abstract

Objective UV-A/Riboflavin cross-linking of corneal collagen fibers (CXL) is a highly promising therapy for corneal melting in humans. A prospective interventional, nonrandomized, controlled study was conducted to compare the stabilizing effect of CXL treatment on melting keratitis in dogs and cats and the complication rate of CXL to those of standardized intensive medical treatment.

Procedures Forty-nine eyes with melting keratitis were included in the study between October 2009 and October 2012. All eyes were treated according to the same medical treatment protocol. Nineteen eyes were CXL-treated, and 30 eyes were not. Follow-up included slit-lamp examination, fluorescein staining, ulcer size measurement, stromal stability evaluation, photographic documentation, and documentation of complications.

Results Five of 19 eyes in the CXL group and 9/30 eyes in the control group required rescue stabilization due to continued melting. Seven of the nine control group corneas stabilized after rescue CXL treatment. At initial presentation, the ulcers in the canine CXL group were significantly deeper and larger than in the control group. Ulcer deepening during follow-up was more pronounced in the canine control group than in the canine CXL group. CXL treatment-related complications were not observed.

Conclusions Based on the similar failure rates in the control and CXL treatment groups despite the poorer initial situation in the CXL group, the tendency for the ulcers in the control group to deepen and the stabilization of all corneas receiving CXL rescue treatment, we believe that CXL has its place as an adjunctive therapy for melting keratitis in veterinary ophthalmology.

Key Words: cat, cornea, corneal collagen cross-linking, dog, medical therapy, melting keratitis

INTRODUCTION

Melting keratitis or keratomalacia is a serious condition that occurs with relative frequency in veterinary ophthalmology, especially in predisposed breeds.[1–4] Melting keratitis is caused by the release of endogenous and exogenous collagenolytic enzymes and an imbalance between these proteolytic enzymes and the proteinase inhibitors present in the cornea and precorneal tear film.[5,6] Such a release of collagenases can be caused by primary diseases of the ocular surface that weaken the cornea's anatomic barriers and physiologic defenses (like low corneal sensation, quantitative and qualitative tear film deficiencies, exposure keratitis, trauma, eyelid abnormalities, etc.), topical medications, systemic immune-mediated diseases, and secondary bacterial or fungal corneal infections.[7–10]

If uncontrolled, melting keratitis can lead to complete structural disintegration of the cornea, corneal perforation, and eventual loss of the eye.[3,4] Aggressive treatment with topical antimicrobials to battle a potential infection and anticollagenases to directly counter collagenolysis are therefore indicated to stop progression of the melting process.[5] Surgical stabilization of the cornea is indicated when significant progression of the melting process

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despite medical therapy is observed or when the integrity of the globe is significantly compromised at initial presentation.[3] Conjunctival grafts are typically used as they provide tectonic, antimicrobial, and anticollagenase support for a melting ulcer. However, the use of conjunctival grafts exacerbates the corneal opacity, which develops as a result of corneal stromal ulcer healing. Depending on the initial lesion size, depth and localization, the residual visual impairment can be more or less severe.[3,11,12] Another major problem is the potential rapid progression of melting keratitis, which makes timely control over the disease process difficult, both with medical and conventional surgical intervention.

Natural covalent cross-links between the corneal collagen fibers improve the biomechanical stability of the cornea. Cross-linking of corneal collagen (CXL) uses riboflavin (vitamin B2) that acts as a photosensitizer when exposed to UV-A light with a wavelength at the riboflavin absorption peak of 370 nm. This results in a photopolymerization process powered by free oxygen radicals introducing additional cross-links within and between collagen fibers in the corneal stroma up to a depth of 300 μm.[13] The result is an increase in the biomechanical and biochemical stability of the cornea and reactive oxygen species (ROS)-induced damage to cells and microorganisms in the irradiated area.[14–18] In a riboflavin-saturated cornea of ≥400 μm thick, the UV-A irradiance generated at the level of the endothelium with the standard CXL procedure is less than half the endothelial damage threshold. All structures behind a 400 μm-thick corneal stroma, including the corneal endothelium, iris, lens epithelium, and retina, are exposed to a residual UV radiation exposure that is regarded as safe for these structures.[13]

Several groups have demonstrated the antimicrobial effect of CXL against a host of bacterial isolates *in vitro*.[19–21]

Corneal collagen cross-linking was developed to increase the stability and reduce the biodegradation of the corneal collagen matrix in primary and secondary corneal ectatic diseases, most notably keratoconus.[22] However, the properties of CXL induced increased corneal rigidity, decreased susceptibility to collagenase enzymes, and ROS-induced toxicity to microorganisms make CXL an attractive adjunctive therapy for the treatment of melting keratitis.

During the last 5 years, several groups have published studies in humans where CXL was used as an adjuvant treatment in cases where medical therapy had failed to control infectious melting keratitis. In all single cases and small case studies published, CXL led to an arrest of progression of infectious melting.[23–27] In two larger case series with 16 and 40 enrolled patients, the reported success rates were 100 and 85%, respectively.[28,29] In one of these two case series, CXL was successfully used as sole treatment, without the use of antibiotics, to stabilize corneas with confirmed (13 of 16 cases) and presumed (3 of 16 cases) bacterial keratitis.[28]

The use of CXL as an adjunctive therapy for the treatment of melting keratitis may become its major indication in veterinary medicine. We have recently published a pilot study describing the successful use of CXL to treat melting keratitis in three dogs and three cats. Superficial corneal pigmentation, sequestrum formation, and bullous keratopathy were observed during follow-up. It was unclear whether these pathologies were preexisting conditions or complications of the CXL treatment and/or the initial melting keratitis.[30] Hellander-Edman *et al.*[31] have described the successful stabilization via CXL of eight of nine equine corneas with melting keratitis.

Antimicrobial drug resistance of pathogens seems to be an increasing problem in veterinary ophthalmology.[32,33] The treatment of certain drug-resistant microorganisms may be facilitated by the direct antimicrobial effect of CXL.[19]

As far as the authors know, no controlled clinical studies attempting to compare the efficacy of CXL to that of medical treatment for melting keratitis have been undertaken.

Therefore, the objectives of this study were to (i) assess the effectivity of CXL treatment in stabilizing the cornea of dogs and cats with melting keratitis and (ii) to compare the effectivity and complication rate of CXL to those of an intensive standard medical treatment protocol.

MATERIALS AND METHODS

Trial design

A prospective interventional, nonrandomized, controlled study was designed to assess whether CXL treatment of eyes suffering from melting keratitis can decrease the incidence of surgical salvage procedures necessary to stabilize the cornea and of surgical globe removal. The purpose of the study was to test the null hypothesis stating that no difference in outcome exists between the patient group undergoing CXL + medical treatment compared to the control group of patients receiving medical therapy alone.

Animals

Forty-nine eyes (46 animals) with corneal melting were included in this interventional prospective study between October 2009 and October 2012. The entry criteria for inclusion into the study were as follows: (i) species (dog or cat), (ii) clinical diagnosis of keratomalacia/melting keratitis (see pretreatment examination), (iii) complete ophthalmic examination by a board-certified ophthalmologist (BS, SP) or an A/ECVO ophthalmology resident (NG, FM, KV) at initial presentation and all subsequent rechecks, (iv) willingness and ability of the owner to comply with the intensive topical treatment schedule and to return for follow-up examinations. The presence of a corneal perforation or descemetocele or the complete absence of any normal appearing corneal stroma in the ulcer site led to exclusion from the study.

Pretreatment examination

Pretreatment analysis included slit-lamp examination, fluorescein staining, measurement of ulcer size using calipers, photography, cytology, and corneal culture and sensitivity testing. Cytology samples were collected from all animals apart from two dogs in the control group, and two dogs and two cats in the CXL-treated group. Culture and sensitivity samples were collected from all cats and all dogs, apart from one dog in the CXL-treated group. The diagnosis of corneal melting was based on a subjective evaluation of stromal stability/melting activity, including the presence of cellular infiltrates, the perceived stability of the stroma, the presence of changes in corneal contour and ulcer depth, and the presence of malacic corneal material in the ulcer area.

Experimental groups

All patients were treated according to the same standard medical treatment protocol, including the use of topical antibiotics, topical and systemic collagenase inhibitors and, if needed, topical atropine 1% and systemic meloxicam and buprenorphine. Table 1 summarizes the medical treatment protocol. The patients were divided into two groups depending on whether the cornea was CXL-treated or not. Patients in the control group were client-owned animals meeting the entry criteria that were treated with medical treatment alone. Thirty eyes (27 animals: 23 dogs and 4 cats) were enrolled in the control group. Patients in the CXL group were client-owned animals meeting the entry criteria that were treated with medical treatment and CXL. Nineteen eyes (19 animals: 12 dogs and 7 cats) were enrolled in the CXL group. Discontinuation of medical treatment was judged unethical in light of the unknown efficacy of CXL treatment in dogs and cats. Allocation to treatment groups was not performed randomly and depended on owner and clinician preference. Table 2 demonstrates the composition of the study groups.

Table 1. Study protocol medical treatment melting keratitis

Topical antibiotics*	Ofloxacin 0.3% drops†
	Oxytetracycline 1% ointment TID
Anticollagenases‡	K-EDTA 0.36% drops†
	Serum drops†
	Doxycycline 5 mg/kg BID on day 1, once daily afterward
Atropine 1% drops	As needed
Meloxicam	As needed: 0.1 mg/kg on day 1 and 0.05 mg/kg once daily afterward
Buprenorphine	As needed in cats: 0.007–0.014 mg/kg QID

*All animals were treated with topical antibiotics until complete epithelial closure had occurred. If applicable, an alternative antibiotic treatment was chosen based on cytology results and pretreatment with Ofloxacin or Oxytetracycline. Options for patients with cocci identified on cytology: Cephazolin 33 mg/mL or Neomycin/Polymyxin/Gramicidin drops. Options for patients with rods identified on cytology: Tobramycin 0.3% or Moxifloxacin 0.5% drops.

†Eye drops were given every 2 h for 24 h, every 4 h afterward.

‡All animals were treated with topical and/or systemic collagenase inhibitors until the corneal stroma was judged to be stable, based on the semi-objective evaluation of stromal stability/melting activity as described in the Materials and Methods.

BID = twice daily; TID = three times daily; QID = four times daily.

The CXL procedure

Corneal collagen cross-linking was performed as previously described.[30] Briefly, all procedures were performed under general anesthesia with the eye anesthetized topically and positioned in a horizontal plane (Fig. 1). Isoosmolar 0.1% riboflavin drops (freshly mixed 0.5% aqueous riboflavin (Vitamin B2; Streuli, Uznach, Switzerland) and sterile 20% dextran T-500 solutions) were administered to the cornea every 3 min for 30 min. The corneas were then irradiated for 30 min with a 365-nm wavelength ultraviolet A light (irradiance: 3 mW/cm^2, UV-X; Peschke Meditrade, Cham, Switzerland) focused on the corneal surface, while taking care to avoid the corneal limbus.[34,35] Riboflavin solution was applied to the cornea every 3 min during the irradiation period. CXL was performed in the presence of a certain risk of UV-induced cytotoxicity to the endothelium in corneas demonstrating significant loss of corneal stroma.

Post-treatment follow-up

The median available follow-up was 2 (range 0.1–12) months and 3 (range 0.25–22.5) months in the control and CXL groups, respectively. Follow-up included slit-lamp examination, fluorescein staining, ulcer size measurements with calipers, photographic documentation, and documentation of complications during all re-examinations.

Post-treatment examinations were performed during initial hospitalization, at days 7, 14 and 28 after initiation of treatment and at various time points during the long-term follow-up. The primary end point variable to be measured was the occurrence of (or need for) surgical stabilization or removal of the eye, which was interpreted as treatment failure. Surgical intervention was recommended in cases where a significant portion of the residual corneal stromal thickness was lost due to progressive corneal melting during follow-up. Surgical intervention was typically recommended if an additional amount of stroma >20% of the normal thickness of the cornea was lost during follow-up. For eyes in the control group, CXL was offered as 'surgical' stabilization option. The time interval between treatment initiation and the stabilization of the corneal stroma (as determined by the lack of signs of melting, see pretreatment examination), the time interval between treatment initiation and closure of the corneal defect (defect fluorescein negative), and the registration and documentation of complications were secondary end point variables.

Statistical evaluation

Treatment failure/success, gender, and laterality were evaluated using Fisher's exact test for contingency tables. The data for dogs and cats were evaluated separately.

Table 2. Baseline characteristics of the patients

	Dogs		Cats	
Group	Control	CXL	Control	CXL
# Animals	23	12	4	7
# Eyes	26	12	4	7
OD	15	5	3	5
OS	11	7	1	2
Age: median (10–90% percentile)	3.8 (0.7–11)	3 (0.4–13.1)	11.5 (6.9–14)	10 (4–13)
Gender				
F	5	4		1
FS	6	2	1	1
M	9	5	1	1
MN	3	1	2	4
Breed (# animals ≥ 3)				
Pug	9	4		
French Bulldog	2	3		
Shih tzu	3			
Poodle	2	1		
Persian			4	2
European Shorthair				3
Miscellaneous	7	4		2
Brachycephalic	15/23	9/12	4/4	2/7
Concurrent + prior problems				
Distichiasis	6	2		
(Lagophthalmos, macroblepharon, nasal lower lid entropion, caruncle trichiasis)	16	7	1	
Low tear production	7	1		
Corneal pigmentation (Brachycephalic: Pug)	13 (11:8)	5 (4:3)		
Ghost vessels			1	
Systemic disease	3 (diabetes mellitus)	1 (immunodeficiency, optic neuritis)		

OD = right eye; OS = left eye; F = female; FS = female spayed; M = male, MN = male neutered; CXL = corneal collagen cross-linking.

Differences between control and CXL groups regarding age, ulcer depth, ulcer size, interval treatment start to stroma stabilization, interval treatment start to defect closure, stromal thinning at last visit, and length of follow-up were evaluated using the Wilcoxon rank sum (Mann–Whitney *U*) test for unpaired nonparametric data. Differences within groups in ulcer depth at presentation, ulcer depth prior to CXL, and maximal ulcer depth observed

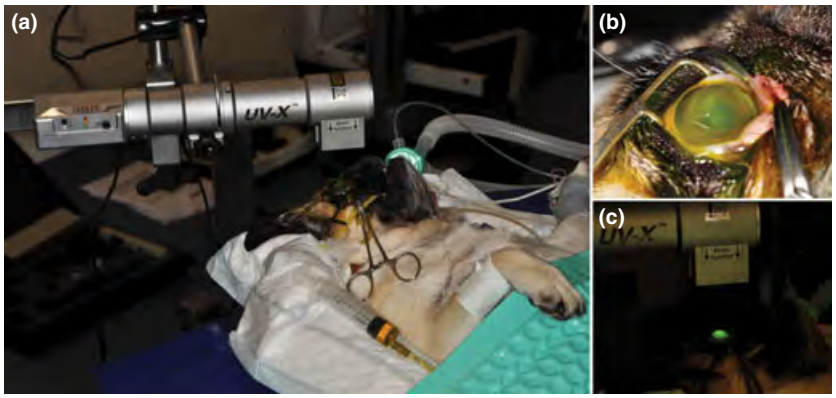

Figure 1. Clinical setup of the corneal collagen cross-linking procedure under general anesthesia. The irradiation source is placed at a distance of approximately 5 cm to the eye (a). The cornea is positioned in a horizontal plane and yellow-colored riboflavin drops are applied (b). The green riboflavin fluorescence is apparent during irradiation at 365 nm (c). The application of fluorescein dye shortly before corneal collagen cross-linking is probably best avoided due to UV-irradiation absorption spectrum overlap of fluorescein and riboflavin.

during the study period were evaluated using the Wilcoxon signed rank test for paired nonparametric data. The level for statistical significance was set at $P < 0.05$ for all comparisons. GraphPad Prism version 6.00 for Windows (GraphPad Software, La Jolla CA, USA, www.graphpad.com) was used for all statistical analyses.

RESULTS

Treatment groups

The number of patients was unequally distributed across treatment groups. Baseline characteristics were well balanced between the canine control and CXL groups with the possible exception of low tear production (<15 mm/min) measured at presentation (Table 2). Brachycephalic animals were equally distributed over and over-represented in the canine control and CXL groups.

The median age of the cats enrolled in the study was 11.5 years for the control group and 10 years for the CXL group. The median age of the dogs in these groups was 3.8 and 3 years, respectively. No significant age difference was found between the control and CXL groups. The right eye was affected more often in cats, and more male cats than female cats were enrolled in the study. All cats in the control group were brachycephalic, whereas only 2/7 cats in the CXL group were brachycephalic.

Clinical features

The numbers of patients with the primary end point (treatment failure/eyes treated) by group, secondary end points, culture results, and complications over a median follow-up of 1.5–5 months are demonstrated in Table 3.

Inflammatory cellular infiltrates were present in all affected corneas, and slit-lamp examination showed loss of corneal stroma in all cases. Significant progression of corneal melting was observed in 9/30 eyes (30%) in the combined canine and feline control groups and 5/19 eyes (26%) in the combined CXL groups. Surgical stabilization was recommended for these eyes, and this was interpreted as failure of the allocated treatment. The median time from treatment start to failure was 2 days in the control group (range 1–24 days) with only two of nine eyes failing treatment after 1 week of follow-up. Median treatment start to failure time in the CXL group was 6 days (range 1–18 days). One eye in the feline control group failed treatment; all other eyes failing treatment were canine eyes. The number of eyes that failed treatment was not significantly different when comparing CXL-treated eyes to eyes that received medical treatment alone in either dogs ($P = 0.71$), cats ($P = 0.36$), or dogs and cats combined ($P = 1$).

A conjunctival pedicle flap was used to stabilize one cornea failing treatment in the control group. Conjunctival pedicle flap placement was strongly recommended for a second control group patient but declined by the owner. Seven eyes of six control group animals were successfully treated with CXL as rescue therapy.

Four of the five corneas failing treatment in the CXL group were stabilized using a conjunctival pedicle flap. A nictitating membrane flap was used in the fifth eye to

Table 3. Clinical results and follow-up

	Dogs		Cats	
Group	Control	CXL	Control	CXL
# Eyes	26	12	4	7
Treatment failure	8/26 (31%)	5/12 (42%)	1/4 (25%)	0/7
Ulcer depth in% at presentation*	35 (10–76)	50 (25.5–70)	55 (20–80)	50 (15–80)
Ulcer area in mm^2 at presentation*	7.5 (2.2–49)	30.5 (4.6–143)	28 (6–96)	144 (30–240)
Interval start treatment – stabilization in days*	3 (0.9–10)	11 (2–45)	7 (3–10)	12 (4–39)
Interval start treatment – defect closure in days*	14 (5.8–37)	33 (13–84)	15 (14–45)	20 (7–60)
Follow-up in months*	1.5 (0.4–7.9)	3 (0.3–7.1)	5 (2–7)	2 (0.5–22.5)
Maximal ulcer depth in%*	50 (14–90)	55 (29–100)	55 (20–80)	60 (15–80)
Stromal thinning at last visit in%*†	20 (9–43)	2.5 (0–50)	10 (0–20)	15 (0–70)
Cytology positive	8	2	1	0
Culture positive	18	5	2	4
Staphylococcus spp (# hem: MRSA/I)	9 (1:2)	2 (1:0)	1	3 (0:1)
Streptococcus spp (# beta-hem)	7 (6)	1	1	1
Pseudomonas spp	1	2		1
Pasteurella spp	2			1
Coliformes	1			
Aerobic sporulating bacteria	1			
Complications				
Progression corneal pigmentation (Brachycephalic: Pug)	11 (8:7)	4 (2:2)		
Endothelial decompensation/Bullous keratopathy	1	1		
Sequestrum			1	2

*Continuous variables are presented as median (10–90% percentiles).
†Estimated as 100 – (stromal thickness at previous ulcer site/thickness normal adjacent cornea × 100).
CXL = corneal collagen cross-linking.

protect a descemetocele during second intention healing. All surgically treated eyes that failed initial treatment were stabilized and retained some form of vision.

One patient with a suspected systemic immunodeficiency was enrolled in the CXL group and failed treatment. Two of three patients with poorly controlled diabetes mellitus, which were enrolled in the control group, failed treatment. One of these two patients was presented in a ketoacidotic crisis with bilateral melting keratitis, and the second patient was suspected of having Cushing's disease.

The ulcer area size was much larger in the CXL group than in the control group in both cats (not significant) and dogs ($P = 0.01$). The ulcer area size was much larger in the cats than in the dogs in both groups. At presentation, the ulcers of patients in the CXL group were deeper than in the control group in dogs ($P = 0.04$), but not in cats. The interval from treatment start to stabilization of the corneal stroma and the interval from treatment start to closure of the epithelium over the defect were longer in the CXL group compared to the control group in dogs ($P = 0.03$ and 0.02, respectively) and cats (not significant). There were no significant differences in the length of follow-up between groups. The maximal ulcer depth observed during follow-up was not significantly different between the control and CXL groups. A significant increase in ulcer depth was observed in both groups in dogs when comparing the ulcer depth at presentation to the maximal ulcer depth observed during follow-up. Ulcer depth increased from a median of 35% to a median of 50% stromal loss in the control group ($P = 0.001$) and from 50% to 55% stromal loss in the CXL group ($P = 0.03$). The differences were not significant in cats. Stromal thinning at the site of the previous ulcer, estimated at the last recorded visit, was more pronounced in the control group (20%) compared to the CXL group (2.5%) in dogs ($P = 0.03$). No difference was observed in cats.

Culture and cytology

One of 11 cat eyes was positive on cytology, compared to 10/38 dog eyes. All cytology positive eyes also yielded positive culture results in both dogs and cats. In dogs, 18/26 (69%) cultures were positive in the control group, compared to 5/11 (45.5%) cultures in the CXL group. One eye in the CXL group had no culture submitted. In cats, 2/4 cultures were positive in the control group, compared to 4/7 cultures in the CXL group. Twenty-five of a total of 34 bacterial isolates were cocci of the genus Staphylococcus or Streptococcus.

Complications

A certain amount of fibrosis was present at the location of the initial ulcer in all eyes, regardless of the treatment group. The density of the fibrosis varied from mild fibrosis which was not obvious to the naked eye, but easily detectable with the use of a slit-lamp biomicroscope at a 10× magnification, to complete opacification of the cornea. The area size affected depended on the area size of the initial ulcer. Appearance of corneal pigmentation (4 eyes) or progression of previously existing corneal pigmentation (7 eyes) was observed in 11/26 eyes (42%) in the canine control group. Eight of these eyes belonged to brachycephalic dogs, and seven to Pugs. Appearance of corneal pigmentation (1 eye) or progression of previously existing corneal pigmentation (3 eyes) was observed in 4/12 eyes (33%) in the canine CXL group. Two of these eyes belonged to brachycephalic dogs, both Pugs.

Dense corneal edema with subepithelial and intrastromal bullae was observed in one dog in the control group and in one dog in the CXL group. Corneal bullae had been observed during wound healing in the cornea of the control group patient. At 3 weeks after treatment start, the cornea was stable and fluorescein negative, and focal edema, neovascularization, and fibrosis were visible. Significant superficial pigmentation, fibrosis, and residual microcystic edema were observed in the cornea from the patient treated with CXL at last recheck at 7.5 months after treatment start.

One of four cats in the control group (Persian) developed a sequestrum 2 weeks after the start of treatment, and 2/7 cats in the CXL group developed a corneal sequestrum during the corneal healing process. The first cat (ESH) developed a sequestrum 2 weeks after CXL, and this sequestrum was spontaneously extruded 3 weeks later. The second cat (Persian) developed a faint brown staining in the superficial stroma at the ulcer site 2 months after CXL. This suspected sequestrum had disappeared at recheck 2 months later. This cat later developed a corneal erosion and similar transient brown staining in the stroma of the fellow eye.

Deviations and violations of protocol

(i) Surgical intervention, constituting treatment failure, was recommended if a loss of more than 20% of the corneal stroma was observed in addition to the stromal loss at presentation. In some cases, an exception was made to that rule. One eye demonstrating a progression from 70% to 80% stromal loss failed treatment in the CXL group. Surgical intervention was recommended for this patient because of a significant increase in ulcer area size and the presence of an instable looking, heavily infiltrated ulcer bed. One eye that was counted as a treatment success in the CXL group demonstrated ulcer depth progression from 50% to 75% stromal loss before the rest of the stroma was diagnosed as being stable. Due to a massive inflammatory cell infiltration affecting the superficial stroma of the entire cornea at presentation, the examiners were not certain whether the ulcer deepening was a result of progressive melting or merely of sloughing of the cellular infiltrates.

Four eyes with a stromal loss progression ≤20% were counted as treatment failures in the control group. One

eye did not demonstrate ulcer deepening, but the appearance of additional stromal ulcers despite medical therapy instead. Two eyes with an additional stromal loss of 10 and 15%, respectively, demonstrated ulcer deepening and a sudden protrusion of central ulcer bed stroma within 1 day. One eye demonstrated an additional loss of 20% of stroma, significant inflammatory cell infiltration of the ulcer bed, the persistent presence of coccoid bacteria on repeated cytology samples, and the appearance of a lipid flare.

(ii) Serum treatment was discontinued shortly after CXL in one cat due to patient compliance problems and concerns regarding the sterility of the dropper bottle nozzle. One dog in the CXL group did not receive topical serum, nor systemic doxycycline treatment. One dog in the CXL group received topical chloramphenicol treatment in addition to the medical protocol. CXL treatment was successful in these three patients.

CXL rescue therapy

Seven eyes of six animals that failed medical therapy were successfully treated with CXL as rescue therapy. Significant ulcer deepening from 30% (median) stromal loss at first presentation to 60% (median) immediately prior to CXL ($P = 0.03$) had been observed. These patients were censored, and the follow-up data presented in Table 4 was not used for the study. Interestingly, ulcer depth did not significantly progress after CXL (Fig. 2b–e), and all seven corneas were stabilized. Follow-up time and the time intervals between CXL and stabilization of the stroma and defect closure were similar to those of the patients in the CXL study group. One cat (Persian) underwent CXL after 1 week of medical Tx and developed a sequestrum 2 weeks after CXL.

Table 4. Corneal collagen cross-linking as rescue treatment in patients failing medical treatment

Demographics	
Animals	5 dogs, 1 cat: 7 eyes
Age in years*	2.3 (0.9–11.6)
Laterality	OD: 5, OS: 2
CXL rescue treatment failure	0
Ulcer area in mm^2 prior to CXL*	12 (5–49)
Ulcer depth in% at initial presentation*	30 (15–66)
Ulcer depth in% prior to CXL*	60 (30–70)
Maximal ulcer depth in%*	60 (50–80)
Interval CXL – stabilization in days*	13 (6–24)
Interval CXL – defect closure in days*	22 (13–36)
Follow-up in months*	2 (0.75–12)
Stromal thinning at last visit in%*	25 (0–50)
Cytology positive	3
Culture positive (all performed prior to start med Tx)	6
Staphylococcus spp (MRSA/I isolates)	5 (2)
B-hemolytic streptococci	1

*Continuous variables are presented as median (10–90% percentiles).

DISCUSSION

The study results are difficult to interpret due to two major limitations of this study.

(i) The group size is too small to give the study the statistical power that it needs to identify a potential true difference in treatment efficacy between the groups.

Especially, the low number of enrolled cats was a likely reason for nonsignificance of all statistical comparisons between the feline control and CXL groups. The decision to stop the current nonrandomized trial was made based on a statistical evaluation of the study results at this time. A study with a patient population five times the size of the present study and an identical distribution of patient characteristics and clinical results between groups would still yield a statistically nonsignificant difference between the control and CXL groups. Such a study would take 10 years to complete with the current speed of patient enrollment.

(ii) Selection bias likely played an important role in this study as the distribution of patients between the control and CXL groups was not randomized and not uniform.[36] The patients in the canine CXL group had significantly deeper and larger ulcers at initial presentation compared to those in the control group. This may be the reason for the significantly longer interval from treatment start to stroma stabilization and from treatment start to defect closure in the CXL group compared to the control group in dogs. This conclusion is supported by the results from Price *et al.*[29] who have reported a correlation between infiltrate diameter and area size at presentation and time to infiltrate resolution, with smaller infiltrates clearing up much faster than larger infiltrates.

The fact that patient evaluation prior to and after treatment was performed in an unmasked manner is another limitation of this study with an unknown effect on the outcome.

Some of the results from this study suggest that CXL could be a useful adjunctive therapy for the treatment of corneal melting in veterinary patients.

(i) The number of eyes that failed treatment was not significantly different when comparing CXL-treated eyes to eyes that received medical treatment alone, despite the poorer situation for the CXL patients at initial presentation. (ii) Ulcer deepening during follow-up was more pronounced in the canine control group (from 35% to 50% stromal loss) compared to the canine CXL group (from 50% to 55% stromal loss), although ulcer deepening was statistically significant in both groups. (iii) Seven of the nine eyes that failed medical treatment were successfully stabilized with CXL.

The overall stabilization rate after CXL of 74% in this study was lower than the success rates of 100 and 85% in previous case series of human patients by Makdoumi *et al.* and Price *et al.*, respectively,[28,29] and lower than the success rate of 89% in a small equine case series described by

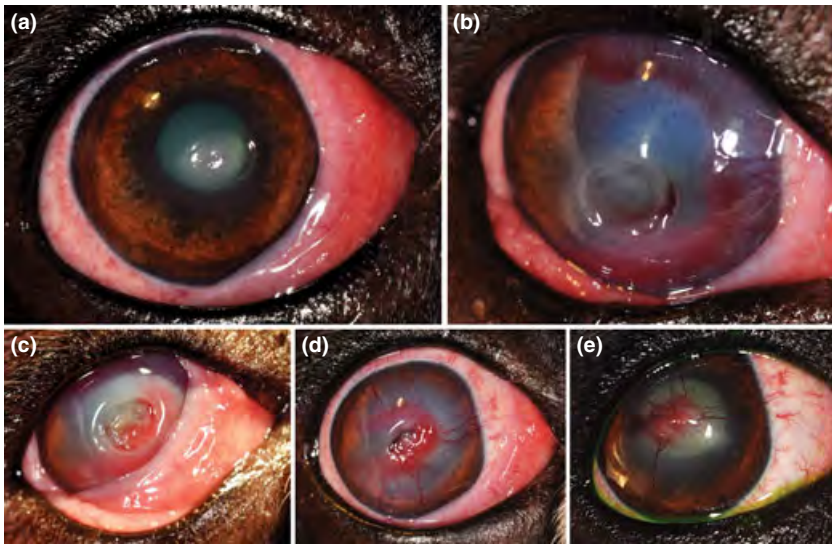

Figure 2. Photographs of the ocular adnexa and cornea of a dog before and after undergoing rescue corneal collagen cross-linking (CXL) treatment. A 2-year-old French Bulldog was treated medically according to study protocol (Table 1) for a melting ulcer OD. After 1 week of treatment, the corneal stroma was still judged to be instable, and 30% of the stroma had been lost at the deepest point of the ulcer (a). During the following 2 weeks, no significant changes were observed despite continued treatment. A sudden rapid deterioration occurred after 3 weeks of treatment, and the dog was presented with a deep, actively melting ulcer. At the deepest point of the ulcer, 60% of the stroma had been lost (b). CXL was performed as rescue therapy and the patient was removed from the study control group. One week after CXL, the ulcer had not deepened further, the ulcer edges were epithelializing and granulation tissue was invading the ulcer bed. Inflammatory cell infiltrates were still present in the central to superotemporal ulcer bed (c). Two weeks after CXL, the ulcer bed was free of inflammatory cell infiltrates, and the ulcer was fluorescein negative. No further ulcer deepening had been observed (d). One month after CXL, the defect was filled with granulation tissue, and the cornea peripheral to the lesion was clearing (e).

Hellander-Edman *et al.*[31] Treatment success was defined as ulcer healing by Makdoumi *et al.* However, an amniotic membrane graft was used after CXL treatment in one patient to reach this goal. Surgical intervention was interpreted as treatment failure in our study and in the studies by Price *et al.* and Hellander-Edman *et al.*

The lower success rate observed in our study may also be explained by the advanced disease state at presentation of most of the ulcers in the CXL group in our study: stromal loss ≥50% in 16/19 ulcers, ulcer diameter range 2.3–13.4 mm (median 6.2 mm). The size of the ulcers ranged between 0.1 and 2.5 mm in diameter (median 1.0 mm) in the study by Makdoumi *et al.*[28] and 0.5 and 12 mm in diameter (median 3.0 mm) in the study by Price *et al.*[29] Infiltrate depth was not a measured data point in either study. However, Price *et al.* observed that infiltrate depth generally increased with increasing infiltrate area. They also noted that after CXL treatment, the disease process resurged within several days after initial stabilization in some cases where infiltrates reached deeper than 50% of the corneal thickness.[29] The same observation was made by Makdoumi *et al.*[28] in one patient with a deep stromal keratitis. They theorized that a corneal infiltrate situated deeper than 300 μm from the corneal surface might well be shielded from the effects of CXL. Whether ulcer depth of more than 50% stromal loss at presentation could be a negative prognostic indicator for CXL treatment is only partially supported by our results. Three of four ulcers that initially presented with >50% stromal loss failed treatment in the canine CXL group, compared to 0/3 in the feline CXL group. Treatment failures in these dogs may be related to a lack of normal cross-linkable stroma in these ulcers. Ulcer depth and area size were not reported for the equine patients of Hellander-Edman *et al.*[31]

Three of four patients with a recognized systemic illness failed treatment in this study, one of which failed medical treatment, but was stabilized with CXL rescue treatment. Patients with systemic abnormalities, like diabetes mellitus, ketoacidosis, and Cushing's disease, that have a negative influence on immunocompetence and/or wound healing may have a poorer prognosis regarding corneal ulcer healing compared to systemically healthy patients.[37,38]

The ulcers were larger in the cats, and the cats were older compared to the dogs in both groups in this study. We have no explanation for these differences. Only one of 11 cats (9%) failed treatment compared to 13/38 dogs (38%). The cause for and significance of this difference is unclear but could be related to the different underlying primary causes for melting keratitis in dogs and cats. A brachycephalic facial conformation likely played an important ulcer permissive role in our canine and possibly feline patients.[3,4] Herpesvirus keratitis has also been implicated in cats.[39]

Forty-six to 69% of the submitted culture samples yielded positive test results in this study, and 74% of the culture isolates were cocci of the genus Staphylococcus (45%) or Streptococcus (29%), which is in agreement with previous studies in dogs.[40–42]

Literature descriptions of a lower prevalence of conjunctival and corneal surface bacterial flora in cats compared to other species [41,43] could not be confirmed in the present study.

Eyes with negative corneal cultures were included in the trial, as would be the case in clinical practice. The culture results did not seem to influence or predict the treatment outcome. Six of nine cases failing treatment in the control group were culture positive compared to 20 positive cultures of a total of 30 cultures submitted in that group. Three of five cases failing treatment in the CXL group were culture positive compared to nine positive cultures of a total of 18 cultures submitted in the CXL group.

Three cases of MRSA/I were identified. One MRSA positive ulcer of a cat treated with CXL was stable 4 days after treatment. The MRSA was sensitive to oxytetracycline and doxycycline however. Two dogs that both failed medical treatment were MRSA/I positive. Both ulcers were treated with CXL as rescue treatment, and both corneas were stabilized. However, based on the antibacterial sensitivity test results, topical chloramphenicol treatment for which these MRSA/I were sensitive had been initiated between CXL treatment and stabilization of the stroma in both cases. Therefore, the stabilization of the ulcers in these two eyes cannot unequivocally be contributed to the CXL effect alone as the change in antibiotic treatment might have had a significant impact as well. Direct CXL treatment-related complications have not been observed in this study.

The incidence of progressive pigmentary keratitis after treatment was similar in both groups of dogs. Preexisting corneal pigmentation was present in 13/26 eyes in the control group, and in 5/12 eyes in the CXL group in dogs, ≥80% of which were brachycephalics and ≥ 60% of which were Pugs in both groups. Most of the dogs that demonstrated post-treatment appearance or progression of pigmentary keratitis in both groups were also brachycephalics and Pugs. These numbers are not surprising as chronic keratitis caused by medial canthal trichiasis, lower nasal eyelid entropion, or macropalpebral fissure[44] is a known stimulus for the development of corneal pigmentation and can also be a predisposing factor for the development of melting keratitis, especially in brachycephalic breeds.[3] Eight of nine Pugs in the control group and 3/4 in the CXL group presented with preexisting pigmentary keratitis, which progressed in 7/8 and 2/3 of these dogs, respectively. These numbers correspond to a recent report by Labelle *et al.*[45] who reported pigmentary keratitis in 80.3% of 295 Pugs examined in a large prospective study.

The incidence of post-treatment endothelial decompensation was low in both groups of dogs (one dog in each group). The CXL procedure itself might have led to the endothelial damage in the CXL-treated patient, as the observed pretreatment stromal loss was significant at 60% in this patient. CXL can pose a serious hazard to the endothelium if an insufficiently thick, riboflavin-saturated stromal layer is shielding the endothelium from hazardous levels of UV-A energy.[46] However, more dogs with ulcers of similar depth were treated with CXL in this study and none developed similar symptoms. Melting keratitis is one of the many other potential causes for endothelial decompensation.[3]

The incidence of sequestrum formation was similar in both groups of cats and could be associated with CXL- and keratomalacia-related keratocyte apoptosis.[47]

This seems to be in agreement with the current literature. No specific safety reports have been published on CXL yet. However, a very low rate or absence of significant, sight-threatening complications has been reported in clinical trials registered to gain FDA approval for the use of CXL in humans.[22,48–52]

A prospective interventional, randomized, controlled study accepting only dogs has been started in our clinic to evaluate the effectivity of CXL + medical treatment compared to medical therapy alone. Power calculations based on published results from human[28,29] and equine[31] case studies and the results of the trial described in this manuscript predict a time frame of at least 3–5 years for this randomized trial to be completed. The authors therefore felt that publication of the results of the present study, especially regarding the lack of observed CXL-related complications, would benefit the veterinary ophthalmic community.

Based on the similar failure rates in the control and CXL treatment groups despite the poorer situation in the CXL group at initial presentation, the tendency for the ulcers in the CXL group to show less deepening and the stabilization of all corneas that received CXL rescue treatment, the authors believe that CXL has its place as an adjunctive therapy for the treatment of melting keratitis in veterinary ophthalmology.

DISCLOSURE

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