

Repair of severe melting ulcers with high intensity UV-pen (18 to 60 mW/cm²) corneal cross-linking (CXL) and amniotic membrane graft

ABSTRACT

Aims: To assess the efficacy of corneal collagen cross-linking (CXL) combined with amniotic membrane graft in the management of severe melting ulcers in the dog and cat using a UV-pen at high intensity.

Study Design: CXL for animal ulcers.

Place and Duration of Study: Kent, UK between June, 2016 and Dec. 2017; and between Feb, 2018 and April, 2018,

Methodology: Medical records of dogs and cats diagnosed with severe melting ulcers, managed with corneal CXL and Omnigen® graft during 2016 and 2017 were retrospectively reviewed. A specially designed small spot size (3 to 10 mm) UV-pen (CXL-100-PEN; made by New Vision Inc, Taiwan) with intensity range of 35 to 60 mW/cm² was used for localized treatment. Following surgical preparation of the recipient cornea, the corneas were soaked with 0.1% riboflavin in 20% Dextran for 15 minutes followed by UV-A (365 nm) irradiation for 60 to 120 seconds, depending on areas treated. Following CXL, Omnigen® graft (1-3 layers) was sutured over the lesion with 8-0 polyglactin 910 (Vicryl; Ethicon). Ten dogs (10 eyes), and three cats (3 eyes) were included. Canine brachycephalic breeds were over represented (7/10 eyes). All eyes had been treated medically prior to surgery, with post-operative antibiotics based on culture-sensitivity results. Treated lesions included large, deep melting ulcers, with 3 extending to Descemet's membrane.

Results: Mean follow-up time was 8 to 10 weeks (range 8-52 weeks). In all cases corneal melting was arrested, with improved ocular comfort within 3 to 6 days of treatment. In 11 eyes the cornea re-epithelialised within 8-20 days. Corneal perforation occurred in 2 eyes between day 16 to 20. Good visual outcome was achieved in 10 cases; with extensive corneal pigmentation in one case, which still remained visual.

Conclusion: CXL combined with an Omnigen® graft is a valuable technique for managing severe keratomalacia, resulting in a good visual outcome compared to traditional grafts.

Keywords: Corneal crosslinking; corneal ulcers; corneal keratoconus; efficacy; riboflavin; ultraviolet light; animal study;

1. Introduction

A melting ulcer is an ophthalmic urgency, which usually progresses in a very short period of time and, if not stabilised promptly, leads to corneal perforation. Corneal melting represents an imbalance between the endogenous and exogenous collagenolytic enzymes and proteinase inhibitors present in the corneal and the precorneal tear film, which effectively lead to the destruction of the collagen of the cornea [1,2]. Surgical treatment of melting ulcers is indicated in cases where there is significant progression of the melting process despite medical treatment. This is mainly achieved by application of corneal graft materials although some post-operative complications such as further progression of the melting process and corneal scarring have been encountered postoperatively [3]. Schnitzler

et al.,[4] described corneal collages cross-linking (CXL) as a successful technique to the traditional surgical techniques that arrested melting process in three out of four human eyes. In veterinary ophthalmology Spiess et al.,[5] performed a pilot study of corneal CXL for the treatment of melting ulcers in three cats and three dogs, and observed inhibition of melting within twelve days, while the cornea re-epithelised between seven and forty days post treatment in both groups. Similarly, Famose [2] reported successful outcome following cross-linking procedures in eight canine eyes and ten feline eyes [3] affected by progressive corneal melting refractory to previous medical treatment, where inhibition of the corneal melting was observed within seven days and epithelial healing was observed within fifteen days post treatment. However, there have been no publications in the veterinary peer-review literature comparing traditional surgical techniques for the treatment of corneal melting ulcers with CXL treatment. Similarly, there have been no publications concerning the sole antimicrobial effect of CXL treatment on melting ulcers, as in the reports that have been published to date the corneas had all been treated with topical antibiotics before the CXL [4-6].

Moreover, conventional CXL device (with large spot size of 7 to 12 mm) has limitation on large area symmetric treatments [7]. There is a strong demand for the treatment of localized irregular ulcers, which requires a small spot (3 to 10 mm) CXL device.

In this study, we will retrospectively report the medical records of dogs and cats diagnosed with severe melting ulcers, managed with corneal CXL and Omnigen® graft from 2016 to 2017 at Elham Valley Referrals. A specially designed small spot (3 to 10 mm) UV-pen (CXL-100-PEN; made by New Vision Inc, Taiwan) with high intensity range of 35 to 60 mW/cm² was used for localized treatments. Following CXL, Omnigen® graft (1-3 layers) was sutured over the lesion with 8-0 polyglactin 910 (Vicryl; Ethicon). The CXL efficacy using accelerated CXL (ACXL) will be analysed based on the new formulas and protocol recently developed by Lin [8-12].

2. Methods and Materials

2.1 animal model

Ten dogs (10 eyes), and three cats (3 eyes) were included. Canine brachycephalic breeds were over represented (7/10 eyes). All eyes had been treated medically prior to surgery, with post-operative antibiotics based on culture-sensitivity results. Treated lesions included large, deep melting ulcers, with 3 extending to Descemet's membrane. Following CXL, Omnigen® graft (made by Nu-vision, UK)(1-3 layers) was sutured over the lesion with 8-0 polyglactin 910 (Vicryl; Ethicon). Table 1 shows the pre-CXL conditions of selected cases.

Table 1. Individual case data.

Patient	Duration	Topical a/b prior to surgery	C&S results prior to surgery
Shih Tzu 5y 3m	7 days	Fusidic acid Ofloxacin	<i>Pseudomonas aeruginosa</i> Sensitivity, see note (1)
Shih Tzu 6y	2 days	Chloramphenicol	Coag.- ve <i>Staphylococcus</i> . note (2)

Pug 7y 2m	7 days	Fusidic Acid	A) <i>Pseudomonas aeruginosa</i> , B) β haemolytic streptococcus (Lancefield Group G) ; note (3)
Pug 7y	16 days	Chloramphenicol	<i>Pseudomonas spp.</i> note (4)
Pug 3y 6m	4 days	Chloramphenicol	Coag. -ve <i>Staphylococcus</i> note (5)
DSH 10y	1 4 days	Fusidic Acid	Negative
Pug 16m	2 days	Fusidic acid	Negative
Pug 11m	1 day	Fusidic acid Gentamicin	Negative
Burmese 12Y	23 days	Fusidic Acid	Negative
DSH 12y	2 days	Fusidic Acid	Negative
JRT 13y	25 days	Ofloxacin	Negative
Labrador 14y	10 days	None	Negative
JRT 11y5m	21 days	Ofloxacin	Negative

90 Nites for Sensitivity:

- 91 (1) Quinolones: Ciprofloxacin only Chloramphenicol, Aminoglycosides, Fusidic Acid: R
 92 (2) Quinolones, Chloramphenicol, Aminoglycosides, Fusidic Acid: S
 93 (3) Quinolones: A,B – Ciprofloxacin only , Chloramphenicol: A-R, B-S,Aminoglycosides: A,B, Fusidic
 94 Acid : A,B-R
 95 (4) Quinolones, Aminoglycosides: S. Chloramphenicol, Fusidic Acid: R
 96 (5) Quinolones, Chloramphenicol, Aminoglycosides, Fusidic Acid: S

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99 2,2 Design and treatment

100 A specially designed small spot size (3 to 10 mm) UV-pen (CXL-100-PEN; made by
 101 New Vision Inc, Taiwan; www.nvi-laser.com; patented by J.T. Lin, 2016) with high intensity
 102 range of 35 to 60 mW/cm² was used for localized treatment. Following surgical preparation
 103 of the recipient cornea, the corneas were soaked with 0.1% riboflavin in 20% Dextran for 15
 104 minutes followed by UV-A (LED light source at 365 nm +/- 5 nm) irradiation for 60 to 120
 105 seconds, depending on areas treated, where small spot size approximately 3 to 6 mm was
 106 used for localized treatment limited to the ulcer tissue and avoid the normal tissue. This
 107 localized small spot is particularly useful for irregular ulcers which cannot be easily treated
 108 by conventional large spot (8 to 12 mm) device. For cornea having a deep stromal defect;

deep ulcers and Descemetocelles, we avoided to cross link those due to toxicity for this thin? Corneas, such that cross linking was performed by treating spots around these deep defects. We avoided to cross-link the areas extending to very deep stroma and Descemet's membrane, such that cross linking was performed by treating spots around these defects. However, 1-3 discs of Omnigen were sutured over the areas of thin cornea to provide a structural support. As shown by Fig. 1, the UV-pen is hand- held (or fixed to a stand) and can be used in a scanning mode to cover desired treating area. The UV-pen is operated by battery and hand-held which is idea for animal treatment. When it is used for human keratoconus, it can scan over the topography-guided pattern to achieve customized outcome, such as off-centre or astigmatism. It can be also attached to a slip lamp.

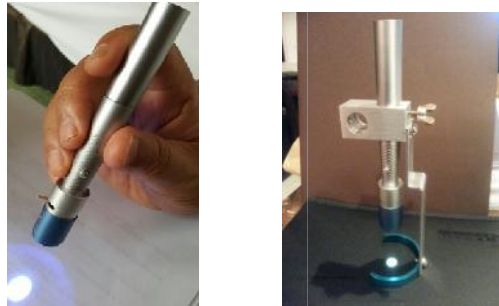


Fig. 1 A CXL-pen (made by New Vision Inc.) with battery-powered and adjustable spot size, operated by either hand-held (left) or a stand (right).

3. Results and Discussions:

3.1 animal data

Mean follow-up time was 8 to 10 weeks (range 8 to 52 weeks). In all cases corneal melting was arrested, with improved ocular comfort within 3 to 6 days of treatment. In 11 eyes the cornea re-epithelialised within 8 to 20 days. Corneal perforation occurred in 2 eyes between day 16 to 20. Good visual outcome was achieved in 10 cases; with extensive corneal pigmentation in one case, which still remained visual.

Examples of 4 cases are shown in Fig. 2, comparing pre-CXL and post-CXL for few days to few weeks.



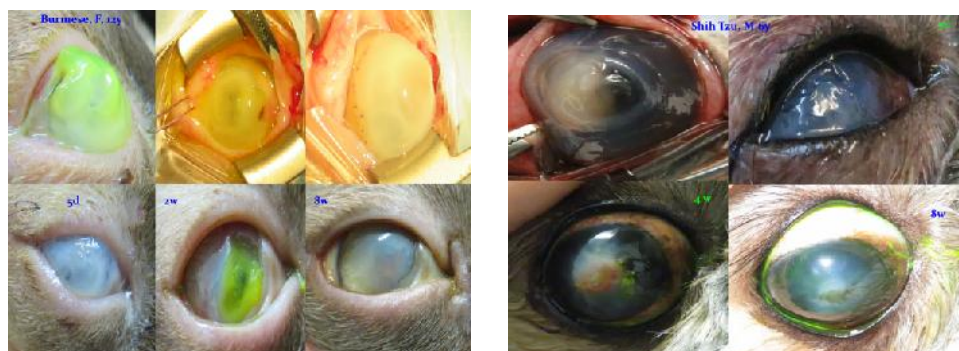


Fig. 2 Examples of 4 cases showing pre-CXL and post-CXL for few days to few weeks.

3.2 Efficacy analysis

To shorten the CXL treatment duration while maintaining the similar CXL efficacy, various accelerated protocols to replace the standard protocol have been proposed based on the Bunsen and Roscoe law (BRL) of reciprocity [7,8] stating that the effect of a photo-biological reaction is proportional only to the total irradiation dose ($E=It$), or the product of intensity (I) and exposure time (t). To achieve the same efficacy, the required exposure time based on BRL is given by $t=E/I$, which gives the protocol for AC; for example, $t=(30, 10, 5, 3, 2, 1.5)$ minutes for $I=(3, 9, 18, 30, 45, 60)$ mW/cm². Validation of BRL for accelerated CXL has been studied by Wernli et al [9] by the cut-off maximum intensity about 50 mW/cm² and a minimum crosslinking time about 2 minutes.

Webb et al [10] report the measured data using Brillouin microscopy for a depth-dependent analysis after CXL. They confirmed the decrease accelerated CXL efficacy was primarily due to the lack of stiffening deeper in the cornea compared with what occurs with the standard (Dresden, 3 mW/cm²) protocol. The cut-off maximum intensity for CXL efficacy were reported as 18 mW/cm² by Hammer et al [11], 34 mW/cm² by Webb et al [9], and 50 mW/cm² by Wernli et al [9]. Unlike findings of Hammer et al and Webb et al, the study of Wernli et al found a relatively constant effect of CXL from 3 mW/cm² through roughly 45 mW/cm². Webb et al [10] interpreted the differences in results could depend on the variability in experimental procedures. Wernli et al kept the corneas immersed in a pool of RF solution for 30 minutes before UVA exposure, whereas in the protocols of Webb et al and Hammer et RF drops were incrementally applied to the cornea. This factor may affect the efficacy, however, we believe that it would be a minor factor, if comparable diffusion depth is achieved in both protocols (after 30 minutes waiting period). Other factors such as the frequency of RF drops applied and their waiting period (for enough diffusion depth) during the UV exposure, could play a more important role [12-16]. More details are discussed as follows. Moreover, extended exposure time based on Lin's non-BRL law, given by $t=\sqrt{\text{sq}(I_0/3)} \times t/\text{BRL}$, may also improve the efficacy [15,16].

The CXL efficacy is influenced by various factors and related to a S-function as $\text{CXL}=1-\exp(-S)$. For type-I CXL, the S-function is given by [12-14] $S1 = K\sqrt{F\text{Co}/(aX\text{Io})} [1 - \exp(-btX)]$, where K is an effective rate constant; $X=\exp(-Az)$; A is an effective absorption approximated by a mean value $A=290(1-0.25z/D)C_0+32$. C_0 is the initial (at $t=0$) Rf concentration (in the stroma) having a diffusion function defined by a diffusion depth (D), $F(z)=1-0.5z/D$. Therefore, high efficacy requires deep Rf diffusion depth (D), high concentration (C_0), but low UV light intensity (I_0). High intensity (or

accelerated CXL, ACXL) suffers low efficacy resulted from its fast Rf depletion, comparing to low, or standard CXL (at 3 mW/cm²).

To overcome the intrinsic drawback of ACXL, Lin [16] recently proposed a new protocol called Rf concentration-controlled method (CCM) to improve the efficacy of ACXL by supplemental Rf (with a frequency defined as Fdrp) during the UV exposure to compensate its depletion by the UV light. The CCM proposed that higher intensity requires larger Fdrop (or more RF resupply) to compensate the faster bleaching effect in the anterior stroma (100 to 250 um) which is retreated by Fdrop times, and the waiting period (with UV off) after each RF drops secures enough diffusing depth (D>150 um).

CXL depth (defined by a maximal S1) is given by $z^* = \ln(NE_0)/A$, with $A = [290C_0 + 32]$, $N = 0.16$ (for $D \gg 500$ um) and 0.224 (for $D = 500$ um) being a numerically fit constant. Therefore, when C_0 is doubled (from 01% to 0.2%), z^* is reduced by 1.48 times. The z^* -formula shows that higher Rf concentrations result in an increased but more superficial cross-linking effect. Therefore, a more appropriate CXL efficacy should be defined by the product of [strength] (or maximal S1) and the [depth] (or z^*), i.e., the volume of stroma being cross-linked. For a given C_0 , deeper CXL may be achieved by larger fluence (E_0).

In this study, much higher intensity (35 to 65 cm²) than the standard CXL (3 to 18 cm²) was used, and no supplemental Rf was applied (i.e., Fdrop=0) during the UV exposure. Therefore, the achieved CXL efficacy was not optimized. To improve the efficacy, we suggest to use iontophoresis for enhanced diffusion depth (with D>250 um). In addition, the CCM should be used. For example, when a UV light intensity 45 cm² is used, having a crosslink time approximately 1 minute, our CCM protocol proposed that after 1 minute UV exposure, turn off the UV and resupply Rf drops, waiting for 1 minute (allowing enough diffusion), then turn on the UV for another 1 minute to finish the crosslink. For more severe melting ulcers, one may repeat the above CCM, such that total exposure time is doubled, and resupply of Rf drops twice (with Fdrp=2) during the UV exposure. Finally, for the similar diffusion depth (D), high Rf concentration (at 0.25%) is always better than 0.1%, as shown by Lin's S-formula, and also clinically reported by O'Brart et al [17] recently.

4. Conclusion

CXL combined with an Omnigen® graft is a valuable technique for managing severe keratomalacia, resulting in a good visual outcome compared to traditional grafts. CXL efficacy may be further improved via CCM and high Rf concentration.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

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