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Repair of Severe Melting Ulcers with High Intensity UV-pen (18 to 60 mW/cm^2) Corneal Cross-linking (CXL) and Amniotic Membrane Graft

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AM, RE, BG performing animal studies. Author JTL device design. Authors MA and JTL discussed, analyzed and prepared the manuscript. All authors read and approved the final manuscript.

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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 30 to 60 mW/cm^2 was used for localized treatment. Following surgical preparation of the recipient cornea, the corneas were soaked with 0.1% riboflavin in 20%

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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 in 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 to10 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 occured in 2 eyes between day 16 to 20. Good visual outcome was achieved in 10 cases; with extensive corneal pigmentation in one case, which stil remained visual.

Conclusion: CXL combined with an Omnigen® graft is a valuable technique for managing severe keratomalcia, 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 corneal melting ulcer is an ophthalmic urgency, usually progresses in a very short period of time and, if not stabilised promptly, leads to deep stromal loss and effectively corneal perforation. Corneal melting represents an imbalance between the endogenous and exogenous matrix metalloproteinases (MMP) and the proteinase inhibitors present in the corneal and the precorneal tear film, which effectively leads to the destruction of the collagen of the cornea [1,2]. This imbalance may be caused by infectious and non-infectious factors. Pseudomonas spp. synthetises two types of exogenous MMP (alkaline proteases and elastase), which are responsible for rapid progression of keratomalcia [2,3]. Other bacteria commonly isolated from infectious melting ulcers are: streptococci and staphylococci [4]. Endogenous MMP are produced by non-infectious factors: keratocytes and inflammatory cells [5]. It has been documented that canine brachycephalic breeds are predisposed to septic melting ulcers, which is related to breed related corneal exposure associated to lagophthalmos, tear film instability and decreased corneal sensitivity [4,6]. Medical management of melting ulcers consists of frequent application of topical antibiotic and anticollagenases [2,3]. Keratomalcia may however rapidly progress, despite the medical treatment, resulting in formation of deep stromal ulcers, Descemetoceles and corneal perforations. 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 a variety of corneal graft materials to stabilize collagen lysis and provide a tectonic support to the weakened tissue [3,6].

Corneal collages cross-linking (CXL) is a new technology, which creates intrafibrillar covalent bonds in collagen fibres of the corneal stroma utilizing a photo-activated riboflavin (vitamin B2) by UV-A light and was introduced to use in human ophthalmology for the treatment of keratoconus in 1999 [7]. Scnitzler et al. [8] used CXL to successfully arrest keratomalcia in three out of four human eyes.

In veterinary ophthalmology Spiess et al. [9] performed a pilot study of 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. Later studies demonstrate satisfactory outcome of accelerated CXL for the treatment of melting ulcers in cats and dogs [10-12].

Amniotic membrane (AM) is a biological grafting material widely used in human ophthalmology for ocular surface reconstruction. AM consists of similar components to the cornea, including laminic and tenascin c, which promote spreading and adhesion of corneal epithelial cells, acting as a biocompatible graft and results in production of transparent tissue [13]. AM acts anti-angiogenic, anti-microbial and inhibits certain MMP [14,15]. AM has also been described in veterinary ophthalmology with a successful outcome in a variety of corneal diseases, including deep corneal ulcers, melting ulcers and corneal perforations [16,17]. Omnigen, a dry human amniotic membrane delivered biological matrix, is an innovation in ocular surface reconstruction as

it is commercially available in a form of discs, easy to store and produces more transparent tissue compared to frozen AM [17].

The conventional CXL procedures (operated at 3 mW/cm^2, for 30 minutes) has been revised to a modern protocol called accelerated CXL using higher UV light intensity (9 to 45 mW/cm^2) to shorten the procedure time based on the Bunsen Roscoe law [18-22].

Moreover, conventional CXL device (with large spot size of 7 to 12 mm) has limitation on large area symmetric treatments [18]. 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 18 to 60 mW/cm^2 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. MATERIALS AND METHODS

2.1 Animal Model

A retrospective study of 10 canine and 3 feline cases presented to the Elham Valley Referrals, UK, between 2016-2017 with severe melting corneal ulcers which were treated with corneal CXL and single or multi-layered Omnigen® graft. Canine brachycephalic breeds were over represented (7/10 eyes). 10 eyes had an area extending to deep stroma and 3 eyes were presented with Descemetoceles. Depth of the lesion was estimated with a slit lamp biomicroscopy. The patients were aged between 11 months and 14 years. All cases were referred for the evaluation after clinical signs deteriorated despite the initial medical therapy. The duration of initial clinical symptoms ranged between 1 and

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	*Pseudomonas aeruginosa Sensitivity, see note (1)*
Shih Tzu 6y	2 days	Chloramphenicol	*Coag.- ve Staphylococcus. note (2)*
Pug 7y 2m	7 days	Fusidic Acid	*A)Pseudomonas aeruginosa, B) β haemolytic streptococcus (Lancefield Group G)* ; *note (3)*
Pug 7y	16 days	Chloramphenicol	*Pseudomonas spp. note (4)*
Pug 3y 6m	4 days	Chloramphenicol	*Coag. -ve Staphylococcus 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

Notes for Sensitivity:

(1) Quinolones: Ciprofloxacin only Chloramphenicol, Aminoglycosides, Fusidic Acid: R

(2) Quinolones, Chloramphenicol, Aminoglycosides, Fusidic Acid: S

(3) Quinolones: A,B – Ciprofloxacin only , Chloramphenicol: A-R, B-S,Aminoglycosides: A,B, Fusidic Acid : A,B-R

(4) Quinolones, Aminoglycosides: S. Chloramphenicol, Fusidic Acid: R

(5) Quinolones, Chloramphenicol, Aminoglycosides, Fusidic Acid: S

25 days and all eyes were treated with topical antibiotics by the first opinion veterinary surgeons, however no bacteriology culture had been carried out prior to antibiotics selection (Table 1). Surgical treatment was carried out under general anaesthesia and with the aid of an operating microscope (Carl Zeiss OPMI Visu 150 S7 Surgical Microscope Germany).

The anaesthetic protocol was same in all cases, consisting of premedication with 0.015 mg/kg IM acepromazine (ACP injection 2 mg/ml Elanco LTD, UK) and 0.3 mg/kg IM methadone (Comfortan ® 10 mg/ml solution for injection, Dechra Veterinary Products, UK), general anaesthesia was induced with 4mg/kg IV propanol (Propofol- Lipuro Vet 10 mg/ml emulsion for injection, Virbac Ltd) and maintained with isoflurane 2% (IsoFlo®, Zoetis, UK) in 100% oxygen. Corneal swab was obtained from diseased corneas and submitted for bacteriology. Cultured revealed bacterial infection in five cases (1 to 5) and was negative in the remaining cases (Table 1). Table 1 shows the pre-CXL conditions of selected cases.

2.2 Design and Treatment

All the corneas were debrided (keratectomy) of the collagen lytic and necrotic tissue with a fine scalpel blade No 64 and/or Castroviejo corneal scissors.

A specially designed small spot size (3 to 10 mm) UV-pen (CXL-100-PEN; made by New Vision Inc, Taiwan; www.nvi-laser.com; patented by JT Lin, 2016) with high intensity range of 18 to 60 mW/cm^2 was used for localized treatment. The focal-distance is approximately 2 to 2.5 cm depending on the spot size. This focal position can be easily defined when the UV spot has a nice circle with sharp edge. We note that the focal distance is a minor factor to the clinical outcomes and safety which are governed by the light fluence on the corneal surface. The current commercial CXL devices have a focal distance from 2 cm to 90 cm, where larger focal distance is easier for surgeon's view, but does not influence the efficacy. Small focal distance might block surgeon's view, but, comparing to a large focal distance, it is less sensitive to eye motion during the procedure.

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 ideal 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.

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 (LED light source at 365 nm +/- 5 mm) irradiation for 60 to 120 seconds, depending on areas treated, where small spot size approximately 3 to 6 mm was used for localized treatment limited to the ulcer tissue and avoid the normal tissue. This localized small spot is particularly useful for irregular ulcers which cannot be easily treated by conventional large spot (8 to 12 mm) device.

CXL treatment was avoided for areas extending to deep corneal stroma and Descemet's membrane. These deep corneal lesions were further treated with application of the Omnigen grafts. Omnigen 1-3 layer graft was placed in the deep corneal defect epithelial side and rehydrated in a sterile saline solution for 2 minutes. The graft then was trimmed to desired shape and size and suture to the cornea with 8-0 polyglactin 910 (Vicryl; Ethicon). Postoperative treatment consisted of broad-spectrum topical until corneas re-epithelised and systemic antibiotics for 10-14 days together with oral meloxicam, 0.3 mg/kg/day (Meloxidyl®, Merial, France).

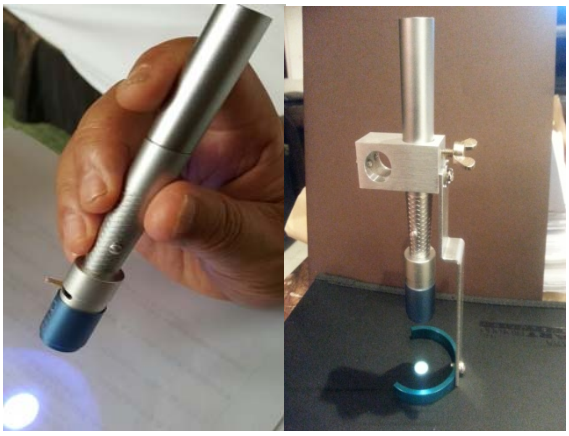

Fig. 1. A CXL-pen (made by New Vision Inc.) with battery-powered and adjustable spot size, operated by either hand-held (left) or a stand (right)

3. RESULTS AND DISCUSSION

3.1 Animal Data

Mean follow-up time was 8 to10 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 occured in 2 eyes between day 16 to 20. Good visual outcome was achieved in 10 cases. An extensive vascularisation of the Omnigen graft occured in one pug (case 7) at 14 days, resulting in the moderate corneal pigmentation. Corneal perforation occured in 2 eyes (case 8 and 3), between day 16 to 20. Examples of 4 cases are shown in Fig. 2, comparing pre-CXL and post-CXL for few days to few weeks. We note that corneal thickness was not measured, because we did not have a pachymetry. Therefore, potential safety issues may exist in some of the treated thin corneas. However, our scanning mode operation over the cornea may reduce the effective dose lower than the Dresden safety dose of 5.4J/cm^2.

3.2 Efficacy Analysis

To shorten the CXL treatment duration while maintaining the similar CXL efficacy, various accelerated CXL [18] protocols to replace the standard protocol have been proposed based on the Bunsen and Roscoe law (BRL) of reciprocity [19] 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/cm^2. Validation of BRL for accelerated CXL has been studied by Wernli et al [20] by the cut-off maximum intensity about 50 mW/cm^2 and a minimum crosslinking time about 2 minutes.

Conventionally believed that the problem of accelerated crosslinking (ACXL) is the lack of oxygen, based on the work of Kling et al [23] that oxygen-mediated type-II CXL plays the critical role of CXL. However, the kinetic model of Kamaev et al. [24] and more recent theory of

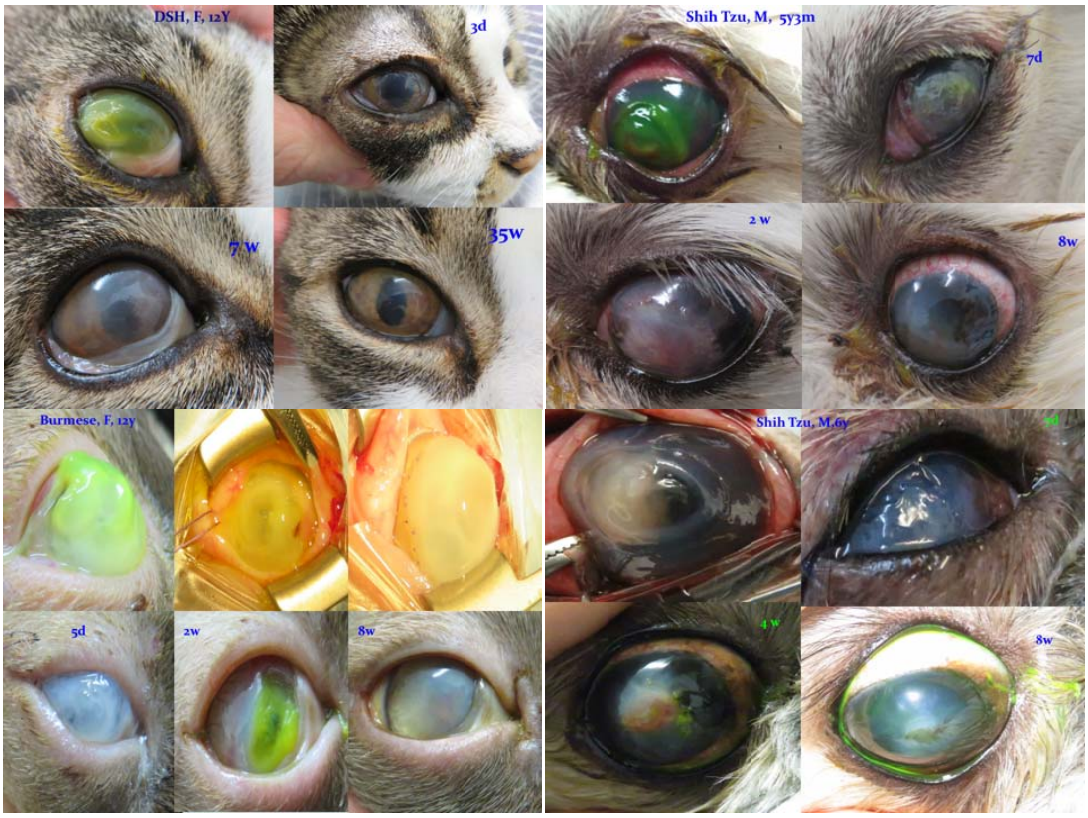

Fig. 2. Examples of 4 cases showing pre-CXL and post-CXL for few days to few weeks.

Lin [25-28] proposed that CXL is predominated by type-I, which does not require oxygen, and it is the predominant pathway of CXL efficacy, while oxygen (in type-II) only plays a limited and transient role. Moreover, Wernli et al [20] found a relatively constant effect of CXL from 3 mW/cm^2 through roughly 45 mW/cm^2. Therefore, the fast depletion of oxygen in ACXL might reduce the type-II efficacy, however, it would not affect the type-I predominant CXL efficacy (which does not require oxygen) [25].

Webb et al. [21] 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/cm^2) protocol. The cut-off maximum intensity for CXL efficacy were reported as 18 mW/cm^2 by Hammer et al [22], 34 mW/cm^2 by Webb et al. [21], and 50 mW/cm^2 by Wernli et al. [20]. 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/cm^2 through roughly 45 mW/cm^2. Webb et al. [21] 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 [27]. More details are discussed as follows. Moreover, extended exposure time based on Lin's non-BRL law, given by t= sqt(I_0/3) x t/BRL, may also improve the efficacy [25].

The CXL efficacy is influenced by various factors and related to a S-function as CXL=1- exp(-S). For type-I CXL, the S-function is given by [25,26] $S1 = K\sqrt{FCo/(bX)}\ [1 - \exp(-0.5btX)]$, where K is an effective rate constant; b=0.62*pk*I_0; X=exp(-*A*z); *p* is the quantum yield of Rf triplet state; *k* is a rate constant; *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/cm^2).

To overcome the intrinsic drawback of ACXL, Lin [27] 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).

In this study, much higher intensity (35 to 65 cm/^{2}) than the standard CXL (3 to 18 cm/^{2}) 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 cm/^{2} 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 [28], and also clinically reported by O'Brart et al [29] recently.

4. CONCLUSION

CXL combined with an Omnigen® graft is a valuable technique for managing severe keratomalcia, 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.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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