Remend®

TAKE A CLOSER LOOK...

REMEND® BIOHANCE™ CROSS-LINKED HYALURONIC ACID

BIOHANCE
CROSSLINKED HA

DÔMES PHARMA

TABLE OF CONTENTS

1

BIOHANCE™ TECHNOLOGY

2

STUDIES WITH BIOHANCE™ CROSS-LINKED HYALURONIC ACID (HA)

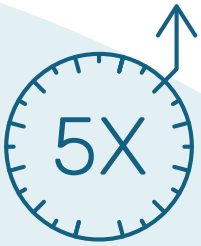
BioHAnce™

TECHNOLOGY

1 HOW DOES A CROSS-LINKED HA COMPARE TO TRADITIONAL HA?

Extended duration on the ocular surface:

Cross-linked HA is more resistant to enzymatic degradation and posesses unique mucoadhesive properties that increases retention time 2-5x longer than traditional, linear HA[1,2]



Improved stabilisation of the tear film:

Cross-linking changes the chemical and physical properties of the HA creating a more viscous lubricant with enhanced mucoadhesive properties, more closely resembling the natural tear film mucins[3]

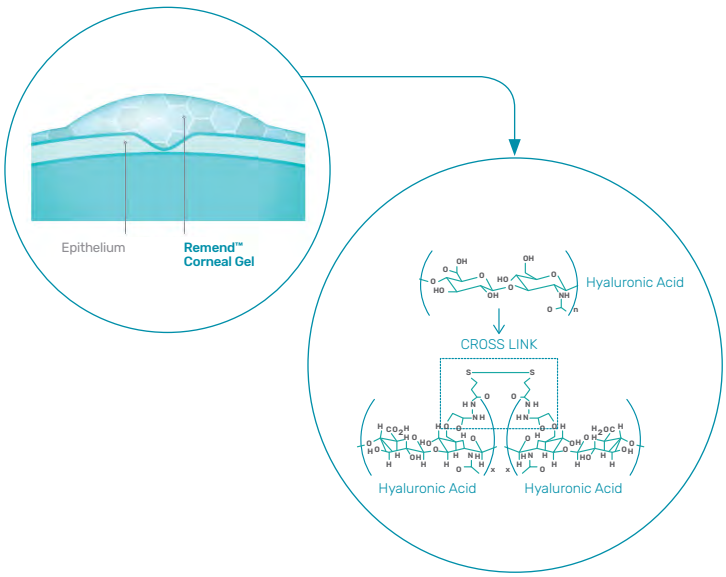
These properties mean that a cross-linked HA provides a multitude of benefits to the patient and owner

2 KEY BENEFITS OF BIOHANCE™ CROSS-LINKED HA

- Enhances hydration, lubrication, ocular comfort and helps stabilise the tear film[3]
- Cross-linked HA creates an environment that supports rapid corneal healing
- Requires fewer applications (BID), and therefore aids pet owner compliance
- Creates a thin barrier that soothes and protects the eye without altering vision
- Shown to prolong the presence of topical treatments on the ocular surface[4]
- Does not bind to nor reduce the efficacy of antibiotics[5]
- BioHAnce™ technology has been created specifically for animal health
- Preservative free

3 REMEND® BIOHANCE™ CROSS-LINKED HYALURONIC ACID

What is Remend® BioHAnce™ Cross-linked hyaluronic acid?



- BioHAnce™ is a patented technology used to create a molecular matrix of cross-linked hyaluronic acid (HA).
- Cross-linking produces unique physical and chemical properties, that enhance hydration, accelerate the body's own healing processes and extend duration on the ocular surface. Remend® Dry Eye Lubricant Drops and Remend® Corneal Gel both consist of BioHAnce™ cross-linked HA.

STUDIES WITH

BioHAnce™

CROSS-LINKED HYALURONIC ACID (HA)

1 EVALUATION OF TOPICALLY APPLIED CROSS-LINKED HYALURONIC ACID (REMEND®) ON THE OCULAR SURFACE OF CLINICALLY HEALTHY DOGS[3]

ACVO 2022 CONFERENCE PROCEEDINGS - SUBMITTED FOR PUBLICATION

Topically applied BioHAnce™ cross-linked HA (Remend®) may improve tear quality, especially tear film stability, in dogs.

(CE Plummer[1], BC Martins[2], C Bolch[3], PS Martinez[1], Carbia BE[1])
1: College of Veterinary Medicine, University of Florida;
2: School of Veterinary Medicine, University of California Davis;
3: Institute for Vision Research, University of Florida
Supported by Bayer Animal Health.

Purpose

To evaluate effects of a topically applied cross-linked HA (Remend® Dry Eye Lubricating Drops) on the ocular surface of clinically normal dogs. Also, to verify the presence and levels of HA in the preocular tear film following topical administration.

Methods

Twenty clinically normal dogs were included. All dogs received a complete ophthalmic examination at day 0, day 7 and day 14 of treatment including:

- Tear ferning test (TF-M7. TF-M5 and TF-R)
- Schirmer's tear test (STT-1)
- Tear film break up time (TFBUT)
- Slit lamp biomicroscopy
- Indirect ophthalmoscopy
- Rose bengal dye staining

On day 0, following examination, dogs began receiving one drop of cross-linked HA (Remend® Dry Eye Lubricating Drops) two times a day (BID) on the right eye. The left eye served as a control and received one drop of isotonic saline BID. The treatment continued throughout the period of the study.

Tear fluid samples from both eyes were collected immediately before and 0.5, 1, 2, 4 and 6 hours after administration. HA levels within the samples were assessed using ELISA.

Results

There was a statistically significant improvement in TFBUT between study and control eyes on Day 7 ($p<0.001$) and Day 14 ($p<0.001$), with treated eyes exhibiting longer tear film stability than control eyes. There was a statistically significant improvement in TF-M7 scores ($p<0.02112$) and TF-R scores by Day 14 ($p< 0.01097$) indicating an improvement in tear quality in treated eyes compared to control eyes. HA was present in measurable quantities in the tear fluid at 30 minutes and one hour after topical application in treated eyes.

2

FLUOROMETRIC EVALUATION OF CROSS-LINKED VS LINEAR HYALURONIC ACID EYE LUBRICANTS[1]

ACVO 2022 CONFERENCE PROCEEDINGS - SUBMITTED FOR PUBLICATION

BioHAnce™ cross-linked HA exhibited a broader ocular surface coverage and a significantly increased ocular surface contact time (up to 180 min) compared with linear HA (up to 36 min).

(F Montiani Ferreira[2], SK Atzet, AD Fankhauser[1], EK Behan[1], DJ Haeussler[3])
1: SentrX Animal Care; 2: Veterinary Medicine Department, Federal University of Paraná; 3: Animal Eye Institute
Supported by SentrX Animal Care.

Purpose

To evaluate the residence time of linear versus cross-linked hyaluronic acid (XHA) on the canine ocular surface, using covalently labeled fluorescent compound.

Methods

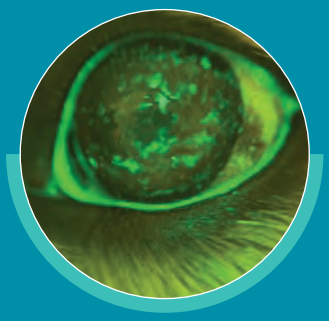
Linear HA and XHA were covalently modified using AlexaFluor 488 reactive moities. Physical properties of the solutions were also evaluated for concentrations, viscosity and shear thinning profiles. Eye drops were applied to eyes of 18 dogs that were previously assessed and determined to have normal baseline ocular health (STT, slit-lamp biomicroscopy, tonometry and fundoscopy). Using a blue light filter (450-490 nm), digital images were obtained, from instillation to 180 minutes. Images were analysed assessing the percent of the total ocular area covered with green fluorescence at various time points.

Results

All HA samples were successfully modified with approximately 5 mol% Alexa-Fluor. Linear HA quickly migrated to the tear meniscus and could be quantified up to 36 min. XHA exhibited a dual phase behaviour: a wide surface coverage first, lasting up to 50 min, then accumulating in tear film meniscus and medial canthus in the second phase, remaining in contact with the ocular surface up to 180 min.

BioHAnce™ cross-linked HA exhibited a dual phase behaviour: a wide surface coverage first, lasting up to 50 min, then accumulation in the tear film meniscus and medial canthus in the second phase, allowing contact with the ocular surface for up to 180 min.

1ST PHASE Wide surface coverage



Remend® BioHAnce™ Corneal Gel (0.75%)

0 min

Remend® BioHAnce™ Corneal Gel (0.75%)

5 min

2ND PHASE Medial canthus deposit phase (slow release)

Remend® BioHAnce™ Corneal Gel (0.75%)

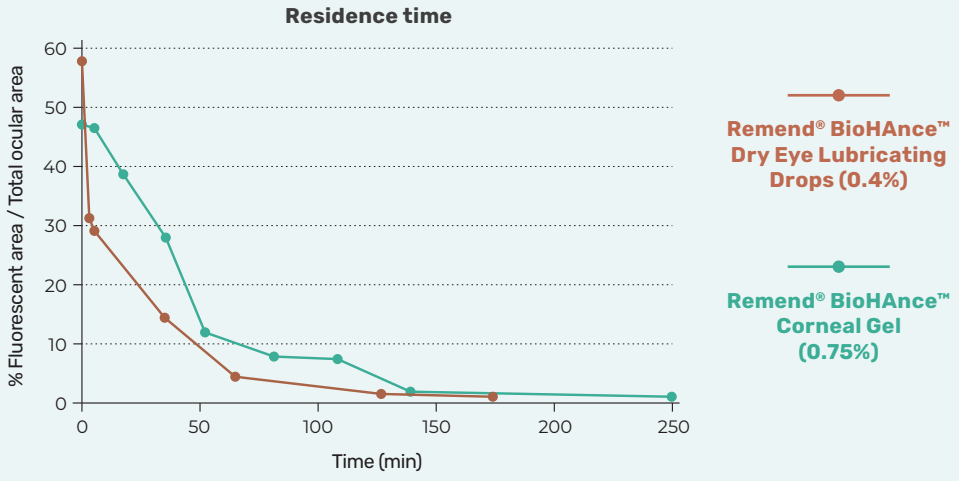
180 min

Everted eyelid showing deposit

Remend® BioHAnce™ Corneal Gel (0.75%)

180 min

Additional privately held study performed by SentrX Animal Care with healthy dog eyes



3 PROOF OF CONCEPT STUDY COMPARING HEALING RATES OF BIOHANCE™ CROSS-LINKED HYALURONIC ACID HYDROGEL VS STERILE SALINE AND AMNIOTIC EYE DROPS

PREPUBLICATION DATA ACVO 2022

BioHAnce™ cross-linked HA showed reduced healing time (30-50%) in comparison to sterile saline drops and amniotic eye drops in a murine model with a standardised corneal epithelial injury.

Purpose

Hyaluronic acid and amniotic-based hydrogels have been shown to enhance corneal healing. The aim of this study was to compare the efficacy of BioHAnce™ cross-linked HA vs sterile saline and amniotic eye drops as well as to understand study group size to power future work.

Materials & Methods

In this model ten rats were anaesthetised via intramuscular injection. They received topical application of 0.4% oxybuprocaine hydrochloride eye drops. During anaesthesia, superficial keratectomy surgery was performed on both eyes.

The size of the corneal epithelial injury was pre-established and performed using a corneal trephine 3.0 mm in diameter. The area bounded by the trephine was then de-epithelialized with a corneal diamond burr, thus generating a central corneal defect, as previously described by Portela 2021[6]. Corneal defects were then evaluated by fluorescein staining and imaged under a blue light immediately following the surgery (time point T=0), then at 12, 24, 36, 48, and 72 hours. Each animal had one eye that served as a control and received sterile saline drops at the same frequency as hydrogel drops. The other eye, the treatment group received 1 drop twice daily of BioHAnce™ cross-linked HA or a commercially available amniotic eye drop.

Results

The cornea was considered healed when there was no fluorescein staining on the corneal surface (Figure 1).

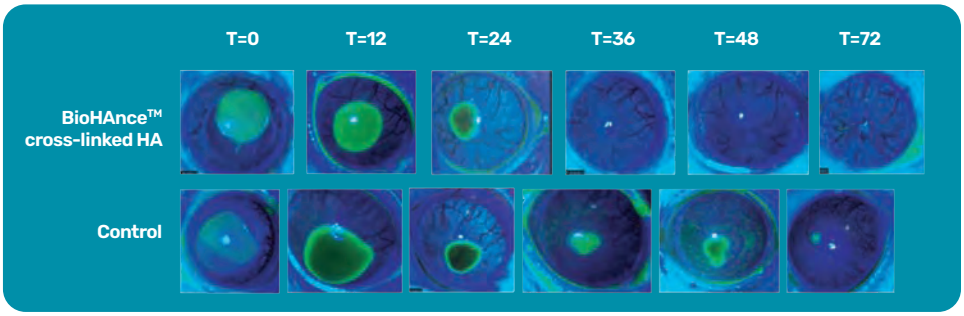


Figure 1. Fluorescein imaging of corneal defects treated with cross-linked hyaluronic acid compared with control (no treatment) immediately following surgery in a rat, T=0 and at time points of 12, 24, 36, 48, and 72 hours.

A paired t-test statistical analysis was performed to compare the control group with the treated group. The treated group had a mean healing time of 48 ± 18 hours and the control group had a mean healing time of 67 ± 11 hours, although in several animals healing was not complete in the control group at the last time point. The healing time was significantly reduced (p=0.048) in the eyes treated with cross-linked hyaluronic acid (Figure 2).

Results cont.

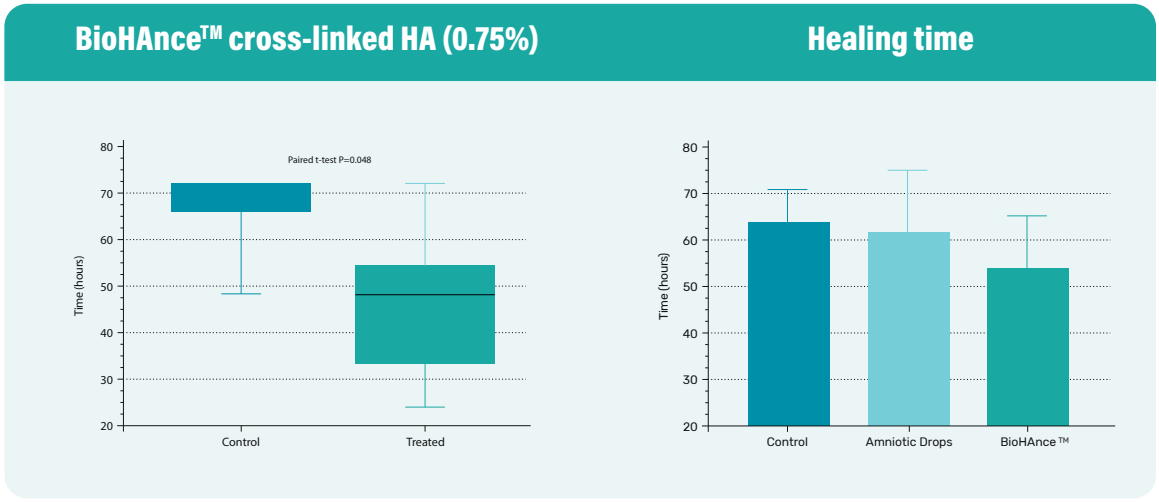


Figure 2. *Box plot of time to healing comparing control (no treatment) with cross-linked hydrogel (treated). Statistical significance from a paired t-test with a p value of 0.048, n=6. Study concluded at 72 hours, so no whisker variation is shown beyond that point.*

Figure 3. *Time to healing comparison of control to two different treatment groups (BioHAnce™ cross-linked HA and amniotic eye drops).*

BioHAnce™ cross-linked HA showed a reduced healing time in comparison to amniotic eye drops (Figure 3). This difference was statistically significant. Furthermore, histopathology indicates that healing with a cross-linked HA appears more organised, with less inflammatory cell infiltrate and closer to normal uninjured morphologies compared to saline or amniotic drops (Figure 4).

This proof-of-concept study supports that BioHAnce™ cross-linked HA contributes to increased healing rates (30-50%) versus control. Indeed, it is also worth noting that measurements for the study were formally stopped at 72 hours while it took over 100 hours to heal the cornea in the control group. This result supports further study into the comparative efficacy of hydrogels on corneal healing time in dogs.

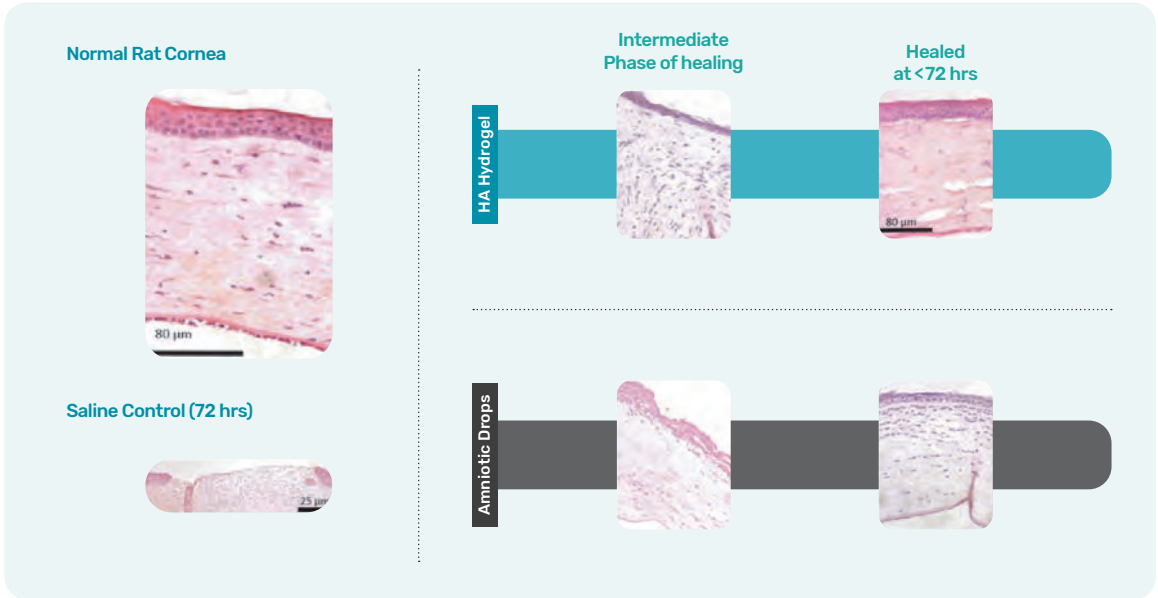


Figure 4. *H&E stained sections of normal rat cornea with no injury, saline treated corneal injury, intermediate phase of healing and fully epithelialised cornea injuries treated with either cross-linked HA hydrogels, BioHAnce™, or amniotic eye drops.*

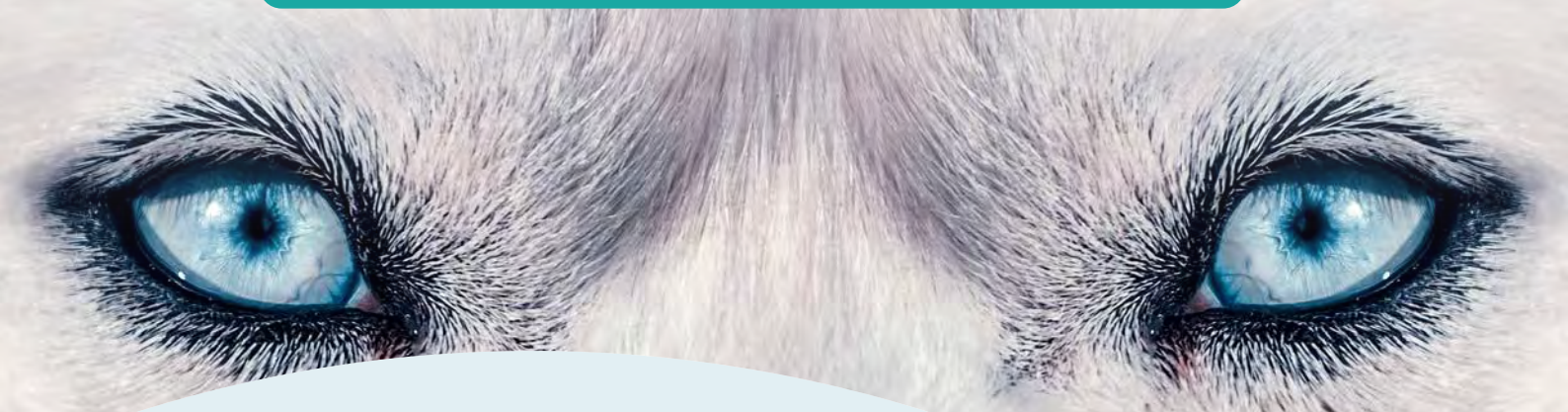
4

TOPICAL CROSS-LINKED HA-BASED HYDROGEL ACCELERATES CLOSURE OF CORNEAL EPITHELIAL DEFECTS AND REPAIR OF STROMAL ULCERATION IN COMPANION ANIMALS[7]

WILLIAMS DL, WIROSTKO BM,GUM G, MANN BK (2017).

INVEST OPHTHALMOL VIS SCI. ; 58:4616-4622.

Remend® BioHAnce™ Corneal Gel administered TID in addition to 0.5% chloramphenicol significantly accelerated the closure of acute corneal stromal ulcers in dogs and cats compared with a linear HA solution. Furthermore, topical administration of the cross-linked HA aided in the resolution of chronic, non-healing corneal ulcers in dogs.



Purpose

The purpose of this study was to determine the tolerance of topical ocular administration of a cross-linked, modified hyaluronic acid (xCMHA-S) hydrogel, and its effectiveness in aiding repair and closure of acute and non-healing corneal ulcers in companion animals.

Methods

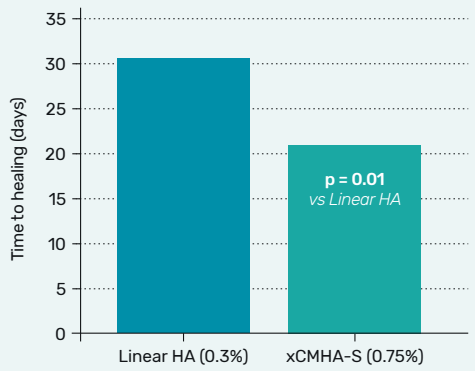
Two concentrations of xCMHA-S (0.4% and 0.75%) were topically administered to the eyes of rabbits six times daily for 28 days to assess tolerance. Then, 30 dogs and 30 cats with spontaneous acute corneal ulcers received either xCMHA-S (0.75%) or a non-cross-linked HA solution (n = 15 per group for each species), three times daily until the ulcer had healed. Finally, 25 dogs with persistent non-healing corneal ulcers received xCMHA-S (0.75%) twice daily until the ulcer had healed.

Results

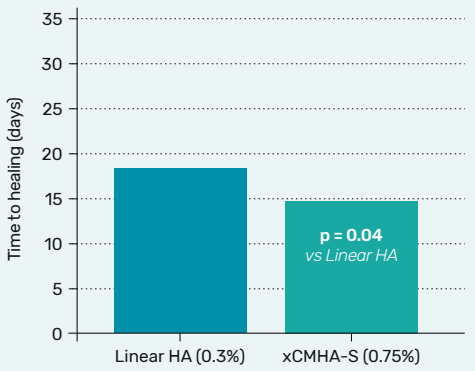
Both concentrations of the xCMHA-S hydrogel were well tolerated and non-toxic in the 28-day exaggerated application study in healthy rabbits. Topically applied xCMHA-S significantly accelerated closure of acute corneal stromal ulcers in dogs and cats compared with a non-cross-linked HA solution. Furthermore, topical administration of the xCMHA-S aided in closure of non-healing corneal stromal ulcers in dogs.

Results cont.

Corneal healing - cats



Corneal healing - dogs



	Remend® BioHAnce™ Corneal Gel	Linear HA	p Value
CATS	n=15 21.0 +/- 11.0 days	n=14 31.8 +/- 10.3 days	0.01
DOGS	n=15 14.8 +/- 4.1 days	n=15 18.3 +/- 4.9 days	0.04

Mean time to healing of acute corneal stromal ulcers in 30 dogs and 30 cats with topical chloramphenicol and three-times daily Remend ®BioHAnce™ Corneal Gel (0.75%) or linear HA

Conclusions

Hyaluronic acid has previously been shown to aid in corneal wound repair. This study demonstrates that a cross-linked, modified HA hydrogel provides additional benefit by accelerating time to corneal wound closure compared to a non-cross-linked HA solution in companion animals.

5

EVALUATION OF CROSS-LINKED HYALURONIC ACID GEL DROPS AND THERAPEUTIC COMBINATIONS FOR OPHTHALMIC INFECTIONS[5]

ACVO 2022 CONFERENCE PROCEEDINGS

The physical properties (viscosity, shear thinning, and concentration) of Remend® BioHAnce™ cross-linked HA are maintained when combined with antibacterial or antiviral drugs. In vitro results suggest that the efficacy of tobramycin is maintained, and that the efficacy of ganciclovir is improved when combined with Remend® BioHAnce™ cross-linked HA.

(SK Atzet[1], AD Fankhauser[1], EK Behan[1], BK Mann[1]) 1: SentrX Animal Care
Supported by SentrX Animal Care.

Purpose

To evaluate the in vitro efficacy and physical properties of combining antibacterial and antiviral drugs with Remend® BioHAnce™ cross-linked hyaluronic acid (XHA) based eye drops.

Methods

Several active pharmaceutical ingredients (Neomycin, Polymyxin B, Bacitracin, Gentamicin, Cefazolin, Ciprofloxacin, Gramicidin, Oxytetracycline, Tobramycin, Cidofovir, and Ganciclovir) were aseptically mixed with XHA, which, with its unique extracellular matrix, was hypothesised to serve both as a delivery vehicle and eye lubricant. The resulting combined hydrogels were then evaluated for changes in physical properties (e.g., viscosity and shear thinning). Tobramycin hydrogels were evaluated for antimicrobial activity using a zone of inhibition assay. Ganciclovir hydrogels were tested for antiviral efficacy using a cytopathic effect assay (CPE) with Feline Herpesvirus 1 (FHV-1). Both were compared with the same drugs diluted in saline serving as controls.

Results

The addition of active ingredients resulted in no significant changes to the viscosity or shear thinning profile of XHA hydrogels. Tobramycin hydrogel and tobramycin control exhibited equivalent zone of inhibition against three strains of bacteria. XHA ganciclovir solution was found to have a 4.3 and 3.2 fold reduction of viral activity as compared with saline solutions of Ganciclovir.

Conclusions

In vitro results suggest that both the unique physical properties (viscosity and shear thinning) of XHA and efficacy of tested active pharmaceutical ingredients are maintained or improved in the case of Ganciclovir. Future work will include target animal efficacy and disease state clinical studies along with application and dosing requirements based on potential synergistic effects from the XHA's increased residence time.

6 CROSS-LINKED HYALURONIC ACID ENHANCES TEAR FILM CONCENTRATIONS OF CEFAZOLIN SODIUM IN CANINE EYES[4]

ACVO 2023 CONFERENCE ABSTRACT - SUBMITTED FOR PUBLICATION

Remend® BioHAnce™ cross-linked HA increased the residency time of antibiotic in the tear film of 10 healthy dogs. The tear film concentration of cefazolin sodium was increased and higher tear film antibiotic concentrations were maintained longer (up to 8 hours) when mixed with Remend® BioHAnce™ Corneal Gel compared to a less viscous eye lubricant.

(L Sebbag, E Ortaeskinaz, Y Goncharov, R Ofri, D Arad) Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel
Supported by SentrX Animal Care

Purpose

It is already established that cross-linked HA's reside on the cornea for longer time periods than linear HA's and less viscous lubricants. It was hypothesised that this could increase the residency time of ocular treatments. The aim of this study was to compare tear film concentrations of cefazolin sodium when formulated with two different ocular lubricants.

Methods

Cefazolin sodium was compounded as a 5% solution using either 1.4% polyvinyl alcohol (PVA) or 0.75% cross-linked hyaluronic acid (XHA; Remend® BioHAnce™ Corneal Gel). Ten ophthalmologically healthy dogs (5 brachycephalic, 5 non-brachycephalics; n = 20 eyes) received one drop of cefazolin-PVA in one randomly selected eye, and one drop of cefazolin-XHA in the other eye. Tear fluid was collected at times 0 min, 1 min, 5 min, 10 min, 15 min, 30 min, 60 min, 120 min, 240 min, 360 min and 480 min. Cefazolin tear concentrations were measured with UV-Vis spectrophotometry.

Results

No significant differences were noted in tear cefazolin concentrations between brachycephalic and non-brachycephalic dogs with either formulation at any time point ($P \geq 0.086$). In all eyes, mean tear film concentrations were significantly higher with XHA than PVA at all time points ($P \leq 0.049$) except for baseline and t = 60-120 min ($P \geq 0.105$). Tear film kinetics of cefazolin-XHA were somewhat 'biphasic', with drug levels decreasing from 0-120 min, then slightly increasing from 120-360 min prior to declining again until the end of the experiment (480 min). The area under the time-concentration curve ($AUC_{0\text{-}480}$) was 2.7 greater with XHA than PVA ($P=0.002$).

Conclusions

XHA greatly improved tear film concentrations of cefazolin sodium when compared with PVA, a less viscous excipient/lubricant. To dictate dosing regimens and determine clinical efficacy, future experiments should assess XHA-cefazolin in dogs with bacterial keratitis and determine clinical breakpoints for cefazolin against common bacterial pathogens of dog eyes.

EYE FOCUS

Dômes Pharma is committed to providing both vets and pet owners with an extensive range of high quality, effective and innovative ophthalmic products, support and services. We help you to take care of your patients - from daily care and prevention to therapeutics.

References

1. **Montiani-Ferreira F, Atzet SK, Fankhauser AD, Behan EK, Haeussler DJ (2022).** Fluorometric evaluation of cross-linked vs linear hyaluronic acid eye lubricants. SentrX Animal Care; Veterinary Medicine Department, Federal University of Paraná; Animal Eye Institute. ACVO 2022.
2. **Bedos L, Allbaugh RA, Roy MM, Kubai MA, Sebbag L. Precorneal retention time of ocular lubricants in dogs.** Iowa State University College of Veterinary Medicine; Koret School of Veterinary Medicine, The Hebrew University of Jerusalem.
3. **Plummer CE, Martins BC, Bolch C, Martinez PS, Carbia BE (2022).** Evaluation of topically applied cross-linked hyaluronic acid (Remend®) on the ocular surface of clinically healthy dogs. College of Veterinary Medicine, University of Florida; School of Veterinary Medicine, University of California-Davis; Institute for Vision Research, University of Florida. ACVO 2022.
4. **Sebbag L, Ortaeskinaz E, Goncharov Y, Ofri R, Arad D. (2023).** Cross-linked hyaluronic acid enhances tear film concentrations of cefazolin sodium in canine eyes. Koret School of Veterinary Medicine, The Hebrew University of Jerusalem, Rehovot, Israel - ACVO Thursday, September 21st, 2023.
5. **Atzet SK, Fankhauser AD, Behan EK, Mann, Brenda K (2022).** Evaluation of crosslinked hyaluronic acid gel drops and therapeutic combinations for ophthalmic infections. Poster session. In: ACVO Conference 2022.
6. **Portela ALBM, Moreno RN, Ribeiro MHML, de Andrade FM, Alves YV, Alves M, Lira RPC (2021).** Role of nicergoline in corneal wound healing in diabetic rats. BMC Ophthalmol. 2021 Feb 9;21(1):77.
7. **Williams DL, Wirostko BM,Gum G, Mann BK (2017).** Topical cross-linked HA-based hydrogel accelerates closure of corneal epithelial defects and repair of stromal ulceration in companion animals. Invest Ophthalmol Vis Sci. ; 58:4616–4622.

Dômes Pharma is the trading name of TVM UK Animal Health Ltd. For further information, please contact Dômes Pharma, Kirtlington Business Centre, Slade Farm, Kirtlington, Oxfordshire, UK. OX5 3JA. T 0800 038 5868. E help@domespharma.com

CFR-030-A4

DÔMES PHARMA

Kirtlington Business Centre, Slade Farm, Kirtlington, OX5 3JA