DocID: https://connectjournals.com/03896.2020.20.4635

Biochem. Cell. Arch. Vol. 20, No. 2, pp. 4635-4639, 2020 | www.connectjournals.com/bca | *ISSN 0972-5075*

HISTOPATHOLOGICAL STUDY OF CORNEAL ULCER HEALING IN RABBITS BY THE EFFECT OF HYALURONIC ACID

Mohammed M. Jassim, Luay Ahmed Naeem* and Zainab Baker Abdul Kareem

Department of Surgery and Obstetrics, College of Veterinary Medicine, University of Basrah, Basrah, Iraq.
*e-mail: luay.naeem@uobasrah.edu.iq

(Received 20 April 2020, Revised 12 July 2020, Accepted 21 July 2020)

ABSTRACT : Corneal ulcer is a common sequence of corneal injuries. Hyaluronic acid used effectively to the ulcer of eye cornea as a result of chemical burn to cause necrotizing of stroma. However, the use of this approach seems to the efficacy of it is too limited for the cases are being seriously. The presented study of use hyaluronic acid for the treatment of moderate necrotizing stromal keratitis (the depth of ulcer less than the half of the corneal stroma) by applied of hyaluronic acid as drops covering in 20 rabbits eyes compared with artificial tear applied on 20 eyes of rabbits. Clinical results showed that the narrowing of corneal ulcer, histological slide appear the re-epithelization of cornea in effected eye of treated group, the corneal re-epithelialization rate after 14 days was higher with Hyaluronic acid compared with other eye drop product (artificial tear). The purpose of our study was to evaluate the efficacy of hyaluronic acid for using to the treating of Rabbit eye suffering from corneal ulcers. The complete corneal re-epithelialization percentage was highest after 14days post debridement with hyaluronic acid to comprise with other products contain artificial tear. The study provide additional evidence results and appeared the potential benefits of sodium hyaluronate (hyaluronic acid) in the recovery of eye and its play a role in the treatment by the rapid restoration of the epithelium of eye. Whilst, additional studies are needed to confirm the use of hyaluronic acid at eye corneal injuries for pets.

Key words : Rabbit eye cornea, corneal ulcer in pets, corneal epithelium damage, dry eye syndrome, healing of rabbit's cornel ulcer.

INTRODUCTION

Corneal ulcer (epithelial defects) is one of the greatest problems in the practice of the ophthalmology. It is may result from one or more conditions causes; such as mechanical trauma, immunological defect and infections, burns by chemical materials, neurogenic, basement membrane disorders and corneal epithelial (Dua and Azuara, 1999 and Pfister, 1992). The classifications of corneal ulcers are depending on the depth of damage (superficial, deep, descemetocele) and facility of healing (refractory, progressive, complicated, and uncomplicated). The ulcer involves the basement membrane with minimal or no stromal involvement and cornea1 epithelium is defined as superficial ulceration. The deep one of ulcerations is extend to one half the stromal depths or more than it, and descemetoceles extend to the level of Descement's membrane layers. The resolving of superficial cornea1 ulcerations that do not within 5 to 7 days are considered refractory and the size or depth are considered complicated of ulcers that progress for healing (Whitley and Gilger, 1999). A conventional treatment for corneal epithelial defect depend on some optional strategies that includes artificial tear drops, therapeutic contact lenses, amniotic membrane transplantation and tarsoraphy, sometimes these strategies may fail to control and manage this problems and need to add some substances or chemical materials that enhance and stimulate the epithelial proliferation and differentiation (Poon *et al*, 2001). The corneal ulcer is regarded one of the following causes and signs it is an idiopathic causes characterized by rapidly progressive, painful and relentless, a chronic ulcerative keratitis regards a peripherally and progresses circumferentially and centrally no associated with scleritis, and no detectable as a systemic disease. It is a progressive destruction of cornea when the diagnosis of exclusion which means all other diagnosable systemic disorders that could be responsible for it and the must be ruled out. Hyaluronic acid is a one of the vitreous humor components as nature material of the eye, there are many successful applications of hyaluronic acid in the ophthalmologic surgery. Hyaluronic acid is used specially as a space filling material in the eye; the active substance sodium hyaluronate is an established active substance described in the European

Pharmacopoeia. The benefits features of the hyaluronic acid is a white or almost white in color, very hygroscopic substance as a powder or a fibrous aggregate which is sparingly water soluble, and virtually in anhydrous ethanol and insoluble in acetone. Hyaluronic acid is plays an important role in development of glycosaminoglycan is a naturally occurring of the extracellular matrix which used in the healing of wound and inflammation (Inoue and Katakami, 1993); therefore, the injection of hyaluronic acid intraocular during surgery play an important role in the maintenances of the shape of the anterior chamber of eye. On the other hand, hyaluronic acid as solu-tions also used to serve as a viscosity-enhancing component in the eye drops and as an cofactor to eye tissue repair (Necas *et al*, 2008).

MATERIALS AND METHODS

Animals

This study was confirmed and approved according to the Basrah Veterinary Medicine Faculty ethics committee. Twenty rabbits (8-12) month's old age weighting (1.5-2 kg) divided randomly in to two groups:

A- Control group (artificial tear)

B- Treated group (hyaluronic acid)

All the rabbits were housed individually indoors beginning 7 days before the first test day. They was exposed to the constant 24h lighting cycle that included 12h of light and 12h of dark conditions and were fed a commercial rabbit diet, and had unrestricted access to water.

Chemical materials

Sodium hydroxide (NAOH) : Sodium hydroxide as liquid characterized in the main features are colorless solution and denser in thickness than water. Direct contact of NAOH may cause severe irritate to the skin, mucous membranes and eye. They consider as toxic material when ingestion of NAOH and tending to cause corrosion to tissues and metals. Prepared the 5% of NAOH to induce corneal ulcer by imbedded the filter paper in the prepared NAOH and applied it on corneal surface to destroyed epithelial layer and induce corneal ulcer.

Hyaluronic acid (HA) : The active substance sodium hyaluronate also known as hyaluronon is naturally produced by the body an established active substance described in the European Pharmacopoeia (Fig. 1). The main features of HA are white in color or almost white, soluble or moderately soluble in water and very hygroscopic powder or a fibrous aggregate in texture.

Fluorescein test : The orange dye of fluorescein used with blue light to detect and diagnosis of foreign body and any damage in the eye and corneal eye damage by direct apply of the fluorescein sticks in the outer layer of eye (Fig. 2). A piece of blotting paper containing the dye will be touched to the surface of eye. The light (blue light) is applied directly on eye, so any defect problems found on the cornea will be stained by the fluorescein dye and take a green color under the blue light, by this technique we can limited and determine the damage and detect the main cause of the cornea problem by measuring

Fig. 1 : Sodium hyaluronate 0.2% (Bausch and lomb)[7].

Fig. 2 : Fluorescein test (Akorn)[8].

the size of lesions with detect the shape of it and location by effect of the staining.

Study design

After prepare the animal for general anesthesia and induce corneal ulcer by applied of NAOH, the control group divided in to two sub group, fifth rabbits for each one as control, while the other ten rabbits of treated group divided into two other group with applied of HA for seventh and fourteen days from study, and then study the clinical and histopathological changes of two groups in different periods (7, 14 days).

RESULTS

A thorough initial visual examination of the eye should be carried out to identify any size or shape abnormality, change in symmetry. Before study the corneal damage must be compare the normal architectural of cornea (Fig. 3) with any abnormality in our study.

Control positive group

Seven days : Histological section of cornea of control group after seven days from burning by NAOH and applied of nature tear (Sterile - Preservative-Free) showed de-epithelization of corneal epithelium in order to increase riboflavin concentration in the *corneal stroma*, as well to corneal debris associated with inflammatory cells infiltration to the site of damage (Fig. 4).

Fourteen days : Histological section of cornea of control positive (14 days) group showed continuity of de-epithelization of corneal epithelium with reducing of

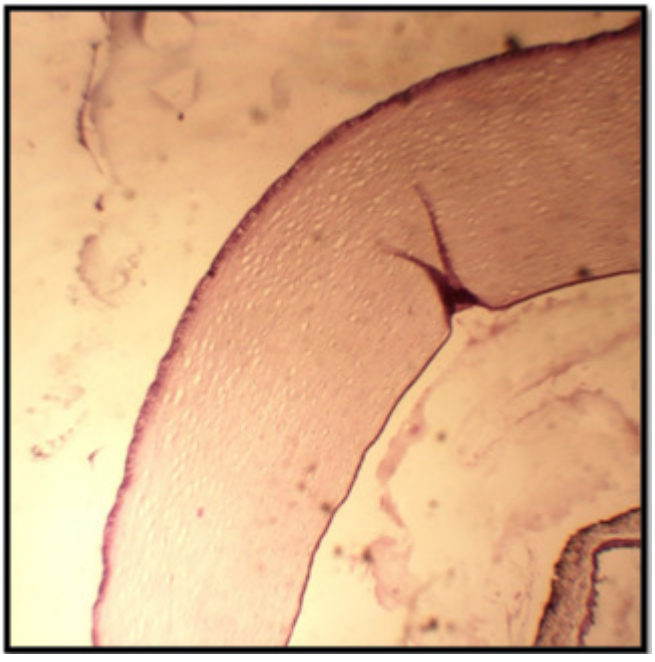

Fig. 3 : Histological section of normal cornea showed normal architectures of corneal epithelium. H&E stain. 10X.

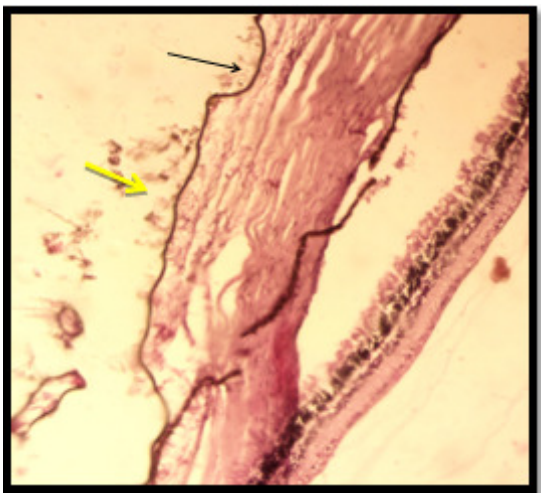

Fig. 4 : Control positive 7 days showed de-epithelization of corneal epithelium (black arrow), with inflammatory cells infiltration (yellow arrow). H&E stain. 10X.

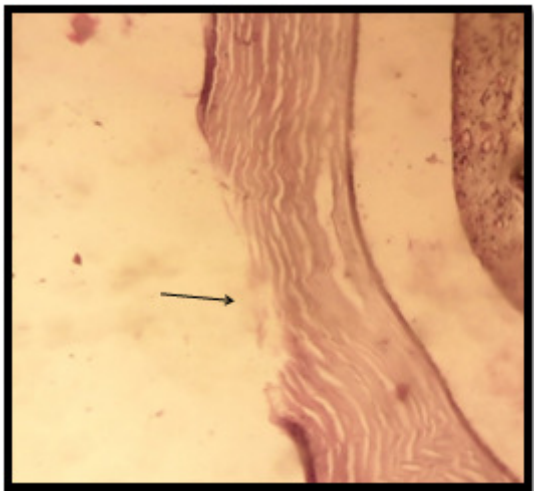

Fig. 5 : Control positive 14 days showed de-epithelization of corneal epithelium (black arrow), H&E stain. 10X

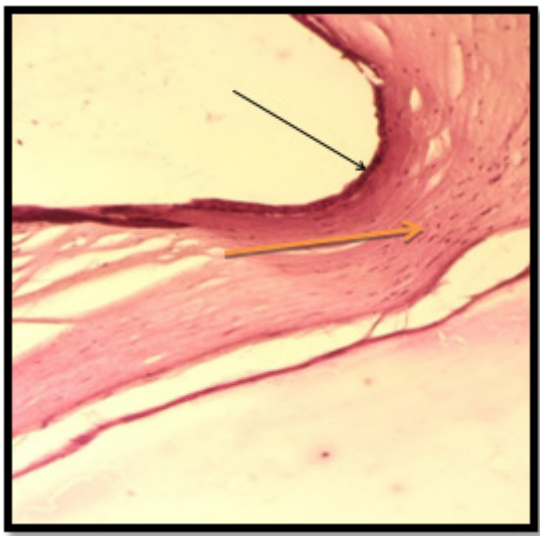

Fig. 6 : Treated group after 7 days showed re-epithelization of cornea (black arrow), inflammatory cells infiltration (orange arrow). H&E stain. 10X

inflammatory cells (Fig. 5).

Treated group

Seven days : Histological section of cornea of treated group after 7 days of applied sodium hyaluronate showed degree of re-epithelization of corneal epithelium as well to invasion of inflammatory cells and infiltration to the site of corneal damage with arrangement of collagen fiber (Fig. 6).

Fourteen days : Histological section of cornea of treated group after 14 days of applied sodium hyaluronate showed complete of re-epithelization of corneal epithelium with invasion of inflammatory cells and infiltration to the site of corneal damage and full degree of arrangement of collagen fiber as normal as possible (Fig. 7).

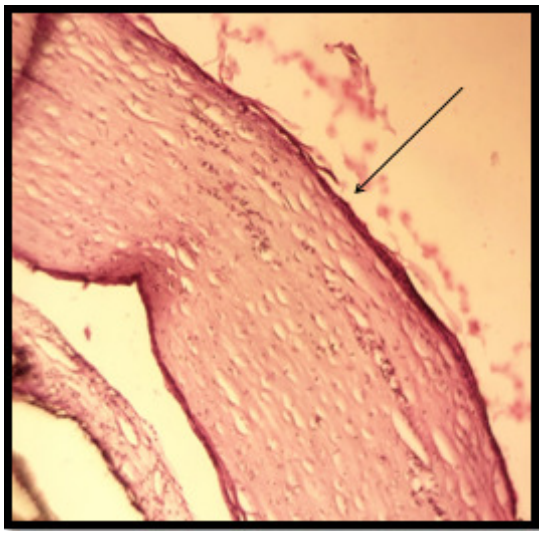

Fig. 7 : Histological section of cornea of treated (14 days) group showed full degree of re-epithelization of corneal epithelium (black arrow) H&E stain.10X

DISCUSSION

The collagen types I, IV and laminin are the nature components of the corneal basement membrane (Grant and Leblond, 1988 and Dua, 1995). Hyaluronic acid is a substance composed in the body naturally. It is a well-known compound of eye drops also at higher concentrations than in the body. Previous studies to compare with our study have the same results appeared that the applied of eye drops containing HA in patients suffering from dry eyes showing improved in the vitality of corneal epithelial cells and increased barrier function (Troiano and Monaco, 2008 and Yokoi *et al*, 1997). Practically there is no sodium hyaluronate or fibronectin in the initiate of injuries in the corneal epithelial, which are only present of it after the process of epithelial lesion healing initiates; sodium hyaluronate and fibronectin these two substances seem to play a role in the migration of epithelial cells that serve as a temporary matrix for the migration during healing of wound (Nakamura *et al*, 1994). During healing process of corneal epithelium and epidermal the sodium hyaluronate (SH) levels are increased when experimentally applied on rabbit's eye by Asari *et al* (1996) and Weigel *et al* (1986). Though, depending on the adhesive property of sodium hyaluronate as mucoid and viscosity features according to the source nature of HA (plant or animal), the contact period time with cornea and bioavailability may differ until at the same concentration of sodium hyaluronate (Baranowski *et al*, 2014; Salzillo *et al*, 2016) that appear clearly in present study in two period of treated group (7 & 14 days). Depending on recent studies to evaluate of HA in vivo and in vitro, these study results showing the HA is known to promote and accelerate healing of corneal wound by prompt corneal epithelial cell to proliferation and migration of inflammatory cells in the lesion site (Nishida *et al*, 1991; Daull *et al*, 2016) this details showing clearly in treated group of our study and the effect is appeared to be concentration dependent and according to the details the sodium hyaluronate can be considered a pure pharmacological effect (Camillieri *et al*, 2004; Daull *et al*, 2016).

The regularity of fibronectin receptors has variation in appearance and disappearance of receptors (integrin) and corneal epithelial cell surface have been shown fibronectin onto during migration (Grushkin-Lerner and Trinkaus-Randall, 1991; Murakami *et al*, 1992; Asari *et al*, 1996).

Its systemic availability will not be significant after application of the eye drops. Overall, efficacy and safety of the active substance in the proposed indications are sufficiently justified. Sodium hyaluronate stimulated corneal epithelial cell migration in vitro, which may have a beneficial effect on corneal wound healing, but to what extent this will be clinically relevant is not known. However, in the study by Camillieri *et al* (2004) there was only a clear effect at concentrations of 0.2% and higher, Gomes *et al* (2004) found no effect on corneal epithelial cell proliferation. Camillieri *et al* (2004) found a stimulating effect on cell proliferation but only at concentrations of 0.2% and higher these results agree with present study to use of hyaluronic acid in the corneal ulcer for re-epithelization.

REFERENCES

Akron.

Asari A, Morita M and Sekiguchi T (1996) Hyaluronan, cd44 and fibronectin in rabbit corneal epithelial wound healing. *Jpn. J. Ophthalmol*. **40**, 18–25.

Baranowski P, Karolewicz B, Gajda M and Pluta J (2014) Ophthalmic drug dosage forms: characterisation and research methods.

Scientific World J. **18**, 861904.

Camillieri G, Bucolo C, Rossi S and Drago F (2004) Hyaluronan-induced stimulation of corneal wound healing is a pure pharmacological effect. *J. Ocul. Pharmacol. Ther.* **20**, 548–553.

Daull P, Feraille L, Elena P P and Garrigue J S (2016) Comparison of the anti-inflammatory effects of artificial tears in a rat model of corneal scraping. *J. Ocul. Pharmacol. Ther*. **32**, 109–118,.

Dua H S (1995) Stem cells of the ocular surface: scientific principles and clinical applications. *Br. J. Ophthalmol*. **79**, 968–969.

Dua H S and Azuara-Blanco A (1999) Allo-limbal transplantation in patients with limbal stem cell deficiency **83**(4), 414-419.

Gomes J A P, Amankwah R, Powell-Richards A and Dua H S (2004) Sodium hyaluronate (hyaluronic acid) promotes migration of human corneal epithelial cells *in vitro*. *Br. J. Ophthalmol*. **88**, 821-825.

Grant D S and Leblond C P (1988) Immunogold quantification of laminine type IV collagen and heparan sulfate proteoglycan in a variety of basement membrane. *J. Histochem. Cytochem*. **36**, 271–283.

Grushkin-Lerner L S and Trinkaus-Randall V (1991) Localization of integrin and syndecan in vivo in a corneal epithelial abrasion and keratectomy. *Curr. Eye Res*. **10**, 75–85.

Inoue M and Katakami C (1993) The effect of hyaluronic acid on corneal epithelial cell proliferation. *Invest. Ophthalmol. Vis. Sci*. **34**, 13–15.

Murakami J, Nishida T and Otori T (1992) Coordinated appearance of beta-1 integrins and fibronectin during corneal wound healing. *J. Lab. Clin. Med*. **120**, 86–93.

Nakamura M, Mishima H and Nishida T (1994) Binding of hyaluronan to plasma fibronectin increases the attachment of corneal epithelial cells to a fibronectin matrix. *J. Cell Physiol.* **159**, 412–422.

Necas J, Bartosikova L, Brauner P and Kolar J (2008) Hyaluronic acid (hyaluronan): a Review Article. *Veterinarni Medicina* **53**(8), 397–411.

Nishida T, Nakamura M, Mishima H and Otori T (1991) Hyaluronan stimulates corneal epithelial migration. *Exp. Eye Res*. **53**, 753–758.

Pfister R R (1992) Clinical measures to promote corneal epithelial healing. *Acta Ophthalmologica* **70** (S202), 73-83

Poon A C, Geerling G, Dart J K, Fraenkel G E and Daniels J T (2001) Autologous serum eyedrops for dry eyes and epithelial defects: clinical and toxicity studies. *J. Ophthalmol.* **85**(10), 1188-1197.

Troiano P and Monaco G (2008) Effect of hypotonic 0.4% hyaluronic acid drops in dry eye patients: a cross-over study. *Cornea* **27**, 1126–1130.

Salzillo R, Schiraldi C and Corsuto L (2016) Optimization of hyaluronan-based eye drop formulations. *Carbohydr. Polym*. **153**, 275–283.

Yokoi N, Komuro A, Nishida K and Kinoshita S (1997) Effectiveness of hyaluronan on corneal epithelial barrier function in dry eye. *Br. J. Ophthalmol*. **81**, 533–536.

Weigel P H, Fuller G M and Leboeuf R D (1986) A model for the role of hyaluronic acid and fibrin in the early events during the inflammatory response and wound healing. *J. Theor. Biol*. **119**, 219–234.

Whitley R D and Gilger B C (1999) Diseases of the canine cornea and sclera. In: *Veterinary Ophthalmology* (ed. gelatt K N), Philadelphia, PA, Lippincott Williams & Wilkins, pp 635-673.