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ORIGINAL ARTICLE

The effect of sub-conjunctival platelet-rich plasma in combination with topical acetylcysteine on corneal alkali burn ulcer in rabbits

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Abstract An experimental study examined the effect of sub-conjunctival platelet-rich plasma (sPRP) in combination with topical acetylcysteine on corneal alkali burn ulcers in rabbits. A total of 20 rabbits were used in this study. After collecting intracardiac blood samples from ten rabbits, platelet-rich plasma was obtained by centrifugation. Alkali wounds were inflicted on the central corneas of rabbits by applying a round filter paper, 6.0 mm in diameter, soaked in 1 M NaOH for 60 s. Only one eye in each rabbit was used. A total of 20 rabbits were allocated into four groups of five animals each. Group 1 served as the control group. Group 2 received 3% *N*-acetylcysteine (NAC) topically three times daily for 2 weeks. The third group received only sPRP whereas group 4 received sPRP with topical 3% NAC three times daily for 2 weeks. Clinical outcome was monitored by evaluation of epithelial defects, corneal opacity, duration of blepharospasm, corneal vascularisation, duration of ocular discharge and wound area diameter measurement. After 3 weeks eyes were enucleated and corneas were excised for histopathological analysis. Samples were assessed by evaluating the number of epithelial rows, stromal vascularisation and inflammation and stromal collagen arrangement. Comparison between groups showed that group 3 had significantly shorter duration of blepharospasm than the control group. Additionally, group 3 had smaller mean defect area and greater wound healing. Histopathlogical investigation revealed significantly less inflammation and vascularisation in the corneas in group 3; this group also had the best stromal collagen arrangement. In conclusion sPRP seems to improve corneal epithelial burn healing. However, acetylcysteine and sPRP combination may have a retarded healing effect as compared with platelet-rich plasma alone.

Keywords Platelet-rich plasma (PRP) · Rabbit · Cornea · Ulcer

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Introduction

Corneal wounds still represent an important problem in clinical ophthalmology because of the loss of transparency of corneal scar tissue (Suzuki et al. 2003).Ocular burns account for 12–19% of eye trauma. This pathology requires quick and efficient action on which the final prognosis will depend (Marquez-de-Aracena et al. 2007). Burns can be chemical or physical. Chemical injuries may cause extensive lesions on the ocular surface and inside the eye (Tsai et al. 2000). Most of chemical burns are produced by acid or alkaline substances. The severity of a chemical burden is related to the concentration of the product, the pH of the solution and the exposure time. Alkaline products cause more serious damage due to the denaturalization of proteins

and saponification of membrane lipids, which allows the alkaline material to quickly penetrate the cornea and enter the eye (Marquez-de-Aracena et al. 2007).

Alkali injuries are more common than acid ones because alkalis are components of the most commonly used cleaning products and civil construction materials (Morgan 1987). Alkalis may cause lesions on the corneal and conjunctival epithelium, basal membrane, keratocytes and stromal nerve endings, corneal and conjunctival vascular endothelium and episclera (Tsai et al. 2000). Alkali-induced corneal lesions frequently result in deep ulcer and perforations (Donzic and Mondino 1987). Despite many clinical treatments being advocated, moderate and severe alkali burns remain difficult to treat and frequently lead to a protracted treatment course with various sight-threatening complications (Abbaszadeh et al. 2009). On the other hand, in corneal ulcers the combination of over expression of certain destructive proteinases and reduction of anti-protease activity can lead to rapid degeneration of collagen and other components of corneal extracellular matrix (Strubbe et al. 2000). Matrix metalloproteinases (MMPs) and serine proteinase seem to be the predominant proteinases in corneal wound healing process. These enzymes are produced by both polymorphonuclear leukocytes and injured epithelial and stromal keratocytes (Chandler et al. 2003). Matrix metalloproteinases inhibitors, such as acetylcysteine (Brown et al. 1970), ethylene diamine tetra acetic acid (Slansky et al. 1969), synthetic inhibitors of metalloproteinases (Byon et al. 1995) and aprotinin (plasmin inhibitor) (Stuart et al. 1989), have been used therapeutically on corneal alkali wounds and have proved efficient in preventing corneal ulceration and perforation. Acetylcysteine has been used for its stability and commercial availability (Berman 1980). However, the need for refrigeration, the frequent instillations required, poor ability to penetrate the corneal stroma and relative toxicity are undesirable characteristic of this agent (Wagoner 1997).

Structural mechanism of wound healing process requires several raw materials such as growth factors, vitamins, glucose and so on. The easiest and most efficacious source to obtain this raw material is blood, as mentioned in other wound healing models. Until recently, various blood derivatives were used for this purpose, such as foetal calf serum, umbilical cord serum and autologous/allogenic serum. Platelet-rich plasma (PRP) is a nontoxic and non-immunogenic product that can easily be obtained from the donor's blood. In the literature, it has been demonstrated that a great amount of growth and wound healing factors were stored as concentrated (Frechette et al. 2005). Growth factors are stored within platelet α-granules, some of them include platelet-derived growth factor, transforming growth factor-ß1, platelet-derived epidermal growth factor (EGF), insulin-like growth factor (IGF) and vascular endothelial growth factor (VEGF). Growth factors are involved in the stages of wound healing and regenerative processes including chemotaxis, proliferation, differentiation and angiogenesis (Bennett and Schultz 1993). Alio et al. revealed that corneal surface application of PRP stimulates and improves corneal epithelial wound healing. Considering the destructive activity of alkali substances on the ocular surface and the consequent direct and indirect intraocular lesions, the purpose of the present study was to investigate the healing process from the effect of 1-shot sub-conjunctival PRP (sPRP) in combination with topical acetylcysteine on corneal alkali burn ulcers.

Materials and methods

This study was approved by the Iranian Laboratory Ethics Committee and carried out under the supervision of Iranian SPCA. Twenty adult female New Zealand Albino rabbits, weighing 2.5–4 kg were used in this experimental study. Before the study all rabbits underwent a complete ophthalmic examination, including indirect opthalmoscopy, slit lamp biomicroscopy, Schirmer's tear test and fluorescein staining. Rabbits with normal eyes were allocated into four groups with five animals each. Animals were anaesthetised with xylazine 2% (5 mg/kg, IMAlfesan, Holland) and ketamine (35 mg/kg, IM Alfesan, Holland). The corneal alkali burn was made by placing a 6-mm diameter, circular piece of filter paper soaked in 1 M NaOH on the central cornea for 1 min. Burning was induced only in the left eye. After disc removal, remnants of the corneal epithelium on the lesion were removed with a sterile swab and the ocular surface was rinsed with 2 ml of physiological saline for 2 min. Fluorescein dye test was used to confirm and delimit the burn lesion induced. A single-dose Flunexin Meglumine (1 mg/kg, sc, Alfesan, Holland) was administered for pain control in all rabbits. Five rabbits without treatment after the alkali burn were designated as the control group. Eyes in group 2 were treated topically with one drop of 3% *N*-acetylcysteine (NAC) (Rotexmedia, Germany) three times a day for 2 weeks (Aldavood et al. 2003). In groups 3 (sPRP group) and 4 (sPRP + NAC) for the preparation of PRP, an 8.7 ml intercardiac blood sample was aspirated (under general anaesthesia) with a 10-ml syringe containing 1.3 ml of Anticoagulant Citrate Dextrose. Each blood sample was centrifuged for 15 min at 72*g* at 4°C resulting in the three following layers: the inferior layer composed of red cells, the intermediate layer composed of white cells and the superior layer made up of plasma. The 6-ml plasma layer was centrifuged for another 5 min at 1,006*g* in order to obtain a two-part plasma: the upper part consisting of 5.5 ml of poor-platelet plasma (PPP) and the lower part consisting of 0.5 ml of PRP. The PPP was first aspirated to avoid mixing up with the PRP. The PRP was then gently

aspirated with another pipette and placed in a sterile tube (Gimeno et al. 2006). Animals in groups 3 and 4 received a single dose of sub-conjunctival autologous PRP. A total of 0.5 ml autologous PRP was injected into the superior bulbar conjunctiva with the aid of 1-ml syringe 27-gauge half-inch needle (Marquez-de-Aracena et al. 2007). One drop of 3% NAC (Rotexmedia, Germany) combination was used, three times daily for 2 weeks in group 4(sPRP + NAC). Low-magnification ophthalmic examination, slit lamp biomicroscopy and the fluorescein dye test were performed at 24-h intervals for 21 days. The wound margin was outlined directly with fluorescein solution, and the eyes were photographed by a digital camera (Olympus DP12, Japan) on days 1, 3, 6, 10, 15 and 21 and the pictures transferred onto a computer. The wound area was measured in square millimetre using an image measurement analysis software (Scion Image, 2000–2001 Scion Corporation, USA). In addition clinical outcome was monitored and documented every day by corneal opacity, duration of blepharospasm, corneal vascularisation and duration of ocular discharge.

Statistical analysis for corneal opacity and corneal vascularisation was performed on days 3, 6, 10, 15 and 21. The severity of corneal opacity was graded 0–3 by an investigator unaware of which group each case came from (Pfister and Pfister 1997). Grade 0 represents a completely clear cornea, grade 1 represents faint corneal haze, grade 2 represents blurred iris detail and grade 3 represents pupil not visible. Corneal vascularisation was graded 0–3 (Kozak et al. 2002). Grade 0 represents no vascularisation, grade 1 represents superficial focal vascularisation, grade 2 represents superficial diffuse vascularisation and grade 3 represents deep vascularisation. At 21 days after alkali corneal burning, each animal's cornea was removed 1 mm close to limbus, and tissue samples were fixed in phosphate-buffered 10% formaldehyde. After fixation of the excised cornea, defective areas were removed and tissue samples were rinsed in phosphate-buffered saline and dehydrated in graded ethanol solutions, cleared in toluene and prepared for routine paraffin embedding. Paraffin sections (5-μm thick) were obtained with Leica SM 2000 R microkeratome and subjected to routine haematoxylin and eosin staining. For each animal, stained sections were examined under the photomicroscope (Olympus DP12, Japan). Histopathological investigations of the sample include five major topics: numbers of epithelial rows, stromal oedema, inflammation reaction and vascularisation and stromal collagen arrangement.

Statistical analysis between the four groups for duration of ocular discharge, duration of blepharospasm, corneal oedema, corneal vascularisation and wound area was performed by Kruskal–Wallis test. Pairwise comparison between each group and control was performed using a Mann–Whitney *U* test. *P* values of <0.05 were considered to be significant.

Results

Freshly burned corneas became cloudy immediately after burning and subsequently turned opaque within 24 h. All rabbits showed blepharospasm due to pain on the first 3 days following ulcer formation and the eyes were semi-closed.

Corneal oedema was most prominent in the first week after surgery in all groups after inducing ulcers. Corneal opacity revealed no significant statistical differences on days 3, 6, 10, 15 and 21 ($P>0.05$).

Comparison between groups showed that the sPRP + NAC group had significantly lower ($P=0.009$) mean discharge days in comparison with the control group. The mean duration of blepharospasm in groups 3 (sPRP group) and 4 (sPRP + NAC) were statistically shorter than the control group ($P=0.009$ in group 3 and $P=0.016$ in group 4) (Fig. 1). Statistical analysis for corneal vascularisation between groups on days 3, 6, 10, 15 and 21 were not significant ($P>0.05$).

There was no significant difference between mean defect area in any groups on days 3, 6, 10, 15 and 21 ($P>0.05$). Generally, comparison between groups showed that group 3 (sPRP group) had smaller mean defect area and greater wound healing (Fig. 2).

Haematoxylin and eosin staining revealed that no eyes were perforated and that Descemet's membrane and endothelium were normal in all 20 eyes. The numbers of epithelial rows in different groups showed no significant statistical difference ($P>0.05$).

Corneal vascularisation revealed significant differences between groups. Pairwise comparison indicated groups 3 and 4 had significantly lower degree of corneal vascularisation than the control group (Figs. 3 and 4) ($P=0.019$).

Stromal oedema was most prominent in the control group and statistical analysis revealed significant differences between groups ($P=0.031$). Pairwise comparison indicated that group 3 had a lower degree of stromal oedema (Figs. 5 and 6).

Statistical analysis showed significant difference between groups in inflammation reaction ($P=0.003$) and group 3

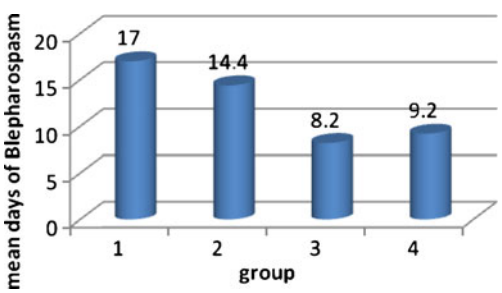

Fig. 1 Bar graph of the mean duration of blepharospasm between the four groups

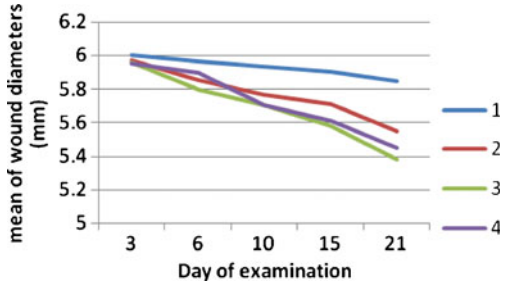

Fig. 2 Line graph of the comparison between the mean defect area in any groups on days 3, 6, 10, 15 and 21

showed significantly less inflammation in comparison with the control group (P=0.005) (Figs. 3, 4, 5 and 6)

Stromal collagen arrangement revealed significant differences between groups (P=0.01). Pairwise comparison indicated group 3 had significantly the best stromal collagen arrangement (P=0.007) (Figs. 5 and 6)

Discussion

The repair of the severely ulcerated cornea is a challenge for ophthalmologists. Alkali burns are the most serious of chemical injuries to the anterior segment of the eye (Meller et al. 2000). Several correction methods and materials have been described in the last few years for defects in the cornea (Abbaszadeh et al. 2009; Chen et al. 2000; Hanada et al. 2001; Kim 2000; Meller et al. 2000; Saberi et al. 2010; Tsai et al. 2000).

The purpose of the present study was to investigate the effect of a 1-shot sub-conjunctival PRP in combination with topical acetylcysteine on corneal alkali burn ulcers healing process.

Acetylcysteine is an MMP inhibitor commonly used in human as well as veterinary ophthalmology (Berman 1980; Kanao et al. 1993; Petroutsos et al. 1982). The beneficial effects of acetylcysteine on corneal wound healing have been reported by several authors (Aldavood et al. 2003; Berman and Dohlman 1975; Berman and Manable 1973; Brown et al. 1970; Burns et al. 1989; Corbett et al. 2001; Fraunfelder et al. 1977; Kanao et al. 1993; Petroutsos et al. 1982). Most of these studies have shown that low concentrations of acetylcysteine, if used topically, have no toxic effects on the cornea, yet accelerate the corneal wound healing (Abbaszadeh et al. 2009; Berman and Dohlman 1975; Petroutsos et al. 1982). However, the need for refrigeration, the frequent instillations required, poor ability to penetrate the corneal stroma and relative toxicity are undesirable characteristic of this agent (Wagoner 1997).

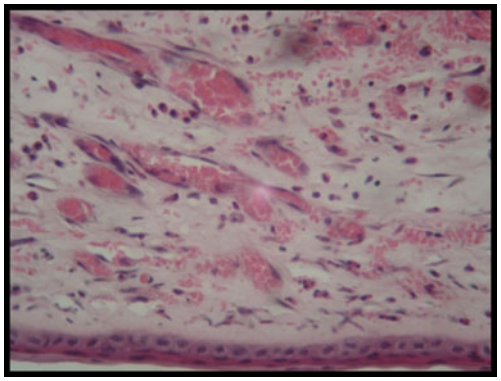

Fig. 4 Histopathological investigations of the sample cornea for group 4 (corneal vascularisation)

Aldavood et al. (2003) evaluated the effects of 3%, 10% and 20% concentration of acetylcysteine on corneal wound healing in dogs. They reported that a 3% concentration of acetylcysteine significantly decreased mean healing time compared with a control group. Petroutsos et al. (1982) studied the rate of re-epithelialization of the rabbit cornea, after receiving isotonic NaCl solution and acetylcysteine (Mucomyst) solution at 10% and 20% concentrations following a superficial epithelial ulcer by fluorescein staining, photography and light microscopy. In this study acetylcysteine solution did not seem to retard normal epithelial healing compared with control animals which received isotonic NaCl solution.

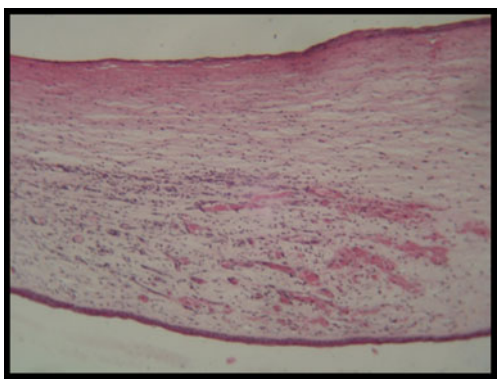

Fig. 3 Histopathological investigations of the sample cornea for group 3 (corneal vascularisation)

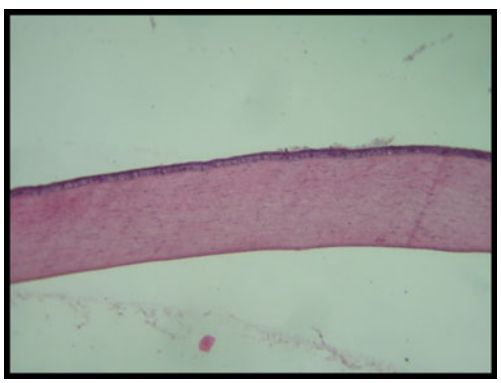

Fig. 5 Histopathological investigation of the sample cornea for group 3 (stromal oedema)

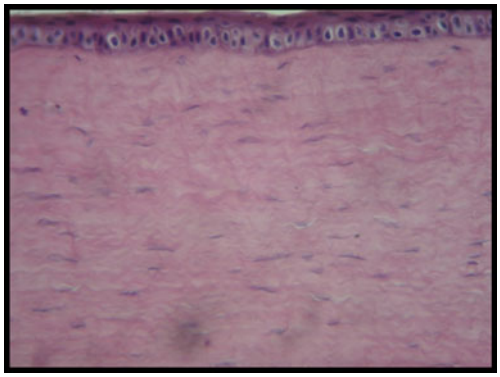

Fig. 6 Histopathological investigation of the sample cornea for the control group (stromal oedema)

Freitas et al. (2003) reported that the autologous blood serum and 10% acetylcysteine did not alter the healing process kinetics of dog corneas that suffered the caustic effects of 3 M sodium hydroxide compared to corneas that received 0.90% balanced saline solution. The authors reported the inefficiency of acetylcysteine in corneal alkali burn ulcers. Likewise in this study, acetylcysteine had no effect on corneal alkali burn ulcer healing when compared with the control group for clinical and histopathological factors. The delay in epithelial healing of the acetylcysteine-treated group may be because of its poor ability to penetrate the corneal stroma and relative toxicity of acetylcysteine (Wagoner 1997). The unique experimental study in the literature investigating the effect of autologous blood derivatives on corneal epithelial wound healing process was undertaken by Akyol-Salman (2006). They used concentrated autologous serum, 20% diluted autologous serum, physiologic saline solution and preservative-free artificial tear drops topically four times a day, each preparation in a different study group, and observed the best results with the concentrated autologous serum group. Autologous serum can provide vitamin A, transforming growth factor-b, fibronectin and other cytokines for ocular tissue (Tsubota et al. 1999). Because PRP contains a large number of whole platelets, they can attach to an ocular surface and accelerate biochemical and biological mechanisms. PRP contains higher concentration of growth factors compared with autologous serum. Frechette et al. (2005) reported that non-activated PRP to whole blood ratio to be 4.6, 1.9 and 3.2 for EGF, IGF-I and angiopoietin-2, respectively.

The major limitation of PRP treatment in animals with low circulation volumes is to obtain enough blood for preparation of the end product. Contrary to the drop form, sPRP can be used as a single shot and does not need any treatment combinations. Thus, we used sPRP instead of drop form because we anticipated better results in rabbits. The first application of sPRP on cornea was undertaken by Marquez-de-Aracena et al. (2007) in patients with ocular burns as a result of work-related accidents. The authors reported a 50% decrease in healing duration after sPRP application as compared with the group treated by conventional procedures.

Alio et al. (2007a, b, c) used PRP in a total of 40 eyes with dormant corneal ulcers in order to investigate the potential role of autologous platelet-rich plasma in promoting healing in dormant corneal ulcers. They reported that PRP improved photophobia, pain and inflammation; facilitated re-epithelialization, promoting corneal wound healing and improving clinical conditions; and resulted in improved vision in the majority of the patients.

Hu et al. (2009) examined the effect of basic fibroblast growth factor (bFGF) on the healing of corneal epithelial wounds in dogs; the histological examination showed more epithelial layers, a more regular rearrangement and fewer inflammatory cells in the epithelium of the bFGF-treated group. Likewise in this study, group 3 (sPRP) had the best result for the clinical and histopathological factors including less duration of blepharospasm, less corneal vascularisation, less corneal inflammation, smaller mean defect area and greater wound healing. Histopathological investigation revealed significantly less inflammation and vascularisation in corneas in group 3. Additionally, group 3 had significantly better stromal collagen arrangement and increased numbers of epithelial rows similar to results from other studies.

Tandir et al. (2011) investigated the effect of single-dose sPRP injections with or without antibiotic treatment on corneal epithelial wound healing in a rabbit model and reported that sPRP seems to improve corneal epithelial wound healing. However, antibiotic and sPRP combination may have a retarded healing effect when compared with PRP alone. They explained that the delay in the epithelial healing of the antibiotic-treated group may be due to the preservative-containing composition of antibiotics.

Interestingly, in the present study, pairwise comparison between groups 3 (sPRP) and 4 (sPRP + NAC) revealed that group 3 had better conditions of wound healing in clinical and histopathological factors (except for mean days of discharge). Perhaps, the frequent instillations required, poor ability to penetrate the corneal stroma and relative toxicity of acetylcysteine were the cause of these results which are similar to those presented by Freitas et al.

The result of this study showed that the subconjunctival application of autologous platelet-rich plasma on corneal alkali burn ulcer is a simple and an economic treatment for ocular surface burns, free of undesirable side effects. It is a safe and easily producible material containing several mediators such as growth factors, which are needed in wound healing. PRP seems to be a promising agent for clinical use in the epithelial wound healing process.

References

Abbaszadeh M, Aledavood SJ, Foroutan AR, Azizzadeh M, SelkGhafai M (2009) Effects of sutureless amniotic membrane patching with 2-octyl cyanoacrylate (Dermaband) on experimental corneal alkali burn in dogs. Comp Clin Pathol 19:357–362

Akyol-Salman I (2006) Effects of autologous serum eye drops on corneal wound healing after superficial keratectomy in rabbits. Cornea 25:1178–1181

Aldavood SJ, Behyar R, Sarchahi AA, Rad MA, Noroozian I, Ghamsari SM, SadeghiHashjin G (2003) Effect of acetylcysteine on experimental corneal wounds in dogs. Ophthalmic Res 35:319–323

Alio JL, Abad M, Artola A et al (2007a) Use of autologous platelet-rich plasma in the treatment of dormant corneal ulcers. Ophthalmology 114:1286–1293

Alio JL, Colecha JR, Pastor S et al (2007b) Symptomatic dry eye treatment with autologous platelet-rich plasma. Ophthalmic Res 39:124–129

Alio JL, Pastor S, Ruiz-Colecha J et al (2007c) Treatment of ocular surface syndrome after LASIK with autologous platelet-rich plasma. J Refract Surg 23:617–619

Bennett NT, Schultz GS (1993) Growth factors and wound healing: biochemical properties of growth factors and their receptors. Am J Surg 165:728–737

Berman MB (1980) Collagenase inhibitors: rationale for their use in treating corneal ulceration. Int Ophthalmol Clin 15:49–66

Berman M, Dohlman C (1975) Collagenases inhibitors. Arch Ophtalmol Rev Gen Ophtalmol 35:95–112

Berman MB, Manable R (1973) Corneal collagenases: evidence for zinc metalloenzymes. Ann Ophthalmol 5:1193–1195

Brown SI, Akiya S, Weller CA (1970) Prevention of the ulcers of the alkali-burned cornea: preliminary studies with collagenase inhibitors. Arch Ophthalmol 82:95–97

Burns FR, Stack MS, Gray RD, CA Paterson (1989) Inhibition of purified collagenase from alkali-burned rabbit corneas. Invest Ophthalmol Vis Sci 30:1569–1575

Byon DS, Kim JC, Shyn KH (1995) The effect of the synthetic inhibitor of metalloproteinase on corneal alkali burn in rabbit. Chung Ang J Med 20:237–252

Chandler HL, Kusewitt DF, Colitz CMH (2003) Enhanced protease production in refractory corneal ulcers. Proceedings of the association for research in vision and ophthalmology. Association for Research in Vision and Ophthalmology, Rockville, p 37

Chen HJ, Pires RT, Tseng SC (2000) Amniotic membrane transplantation for severe neurotrophic corneal ulcers. Br J Ophthalmol 84:826–833

Corbett MC, O'Brart DP, Patmore AL, Marshall J (2001) Effect of collagenase inhibitors on corneal haze after PRK. Exp Eye Res 72:253–259

Donzic PB, Mondino PJ (1987) Management of noninfectious corneal ulcers. Surv Ophthalmol 32:94–110

Fraunfelder FT, Wright P, Tripathi RC (1977) Corneal mucus plaques. Am J Ophthalmol 83:191–197

Frechette JP, Martineau I, Gagnon G (2005) Platelet-rich plasmas: growth factor content and roles in wound healing. J Dent Res 84:434–439

Freitas C, Torrecillhas A, Carmen I, Mendes FA (2003) Ocular alkali lesions in dogs. Acetylcysteine and blood serum effects. Braz J Vet Res Anim Sci 40:36–44

Gimeno FL, Gatto S, Ferro J et al (2006) Preparation of platelet-rich plasma as a tissue adhesive for experimental transplantation in rabbits. Thromb J 4:1–7

Hanada K, Shimazaki J, Shimmura S (2001) Multilayered amniotic membrane transplantation for severe ulceration of the cornea and sclera. Am J Ophthalmol 131:324–331

Hu C, Ding Y, Chen J, Liu D, Ding M, Zhang Y (2009) Treatment of corneal epithelial wounds in dogs using basic fibroblast growth factor. Vet Med 54:280–286

Kanao S, Kouzuki S, Isuneno M, Enomoto H, Yasuhiro K, Yamane Y (1993) Clinical application of 3% *N*-acetylcysteine eye drops in corneal diseases in dogs. J Jpn Vet Med Assoc 46:487–491

Kim JS (2000) Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. Exp Eye Res 70:329–337

Kozak TA, Sevcikova Z, Juhas T (2002) Superficial keratectomy, limbal autotransplantation and amniotic membrane transplantation in the treatment of severe chemical burns of the eye. Acta Vet Brno 71:85–91

Marquez-de-Aracena R, Montero-de-Espinosa I, Munoz M et al (2007) Subconjunctival application of plasma platelet concentrate in the treatment of ocular burns. Preliminary results. Arch Soc Esp Oftalmol 82:475–481

Meller D, Pires RT, Mack RJ (2000) Amniotic membrane transplantation for acute chemical or thermal burns. Ophthalmology 107:980–989

Morgan SJ (1987) Chemical burns of the eye: causes and management. Br J Ophthalmol 71:854–857

Petroutsos G, Guimaraes R, Giraud JP, Renard G, Pouliquen Y (1982) Effect of acetylcysteine (Mucomyst) on epithelial wound healing. Ophthalmic Res 14:241–248

Pfister RR, Pfister DA (1997) Alkali-injuries of the eye. In: Krachmer JH, Mannis MJ, Holland EJ (eds) Cornea. Mosby, St. Louis, pp 1443–1451

Saberi M, Aledavood SJ, Abbaszadeh H, Rezaee M, Azizzadeh M, Ashtari AR (2010) Comparative evaluation in the use of topical corticosteroid in the management of corneal alkali burn ulcer in rabbits. Comp Clin Pathol. doi:10.1007/s00580-010-1140-0

Slansky HH, Gnadinger MC, Itoi M, Dohlman CH (1969) Collagenase in corneal ulcers. Arch Ophthalmol 82:108–111

Strubbe DT, Brooks DE, Schultz GS, Willis-Goulet H, Gelatt KN, Andrew SE, Kallberg ME, MacKay EO, Collante WR (2000) Evaluation of tear film proteinases in horses with ulcerative keratitis. Vet Ophthalmol 3:111–119

Stuart JC, Turgon P, Kowalski RP (1989) Use of aprontin in the treatment of pseudomonas corneal ulceration. Trans Pa Acad Ophtalmol Otolaryngol 41:823–826

Suzuki K, Saito J, Yanai R, Yamada N, Chikama T, Seki K, Nishida T (2003) Cell-matrix and cell-cell interactions during corneal epithelial wound healing. Prog Retin Eye Res 22:113–133

Tandir SF, Yoskel N, Altinats O, Yildiz DK et al (2011) The effect of subconjunctival platelet-rich plasma on corneal epithelial wound healing. Cornea 29:664–669

Tsai RJ, Li LM, Chen JK (2000) Reconstruction of damaged corneas by transplantation of autologous limbal epithelial cells. N Engl J Med 343:86–93

Tsubota K, Goto E, Shimmura S et al (1999) Treatment of persistent corneal epithelial defect by autologous serum application. Ophthalmology 106:1984–1989

Wagoner MD (1997) Chemical injuries of the eye: current concepts in pathophysiology and therapy. Surv Ophthalmol 4:275–313