·Basic Research·

Effect of vasoactive intestinal peptide on the wound healing of alkali-burned corneas

Nese Tuncel [1], Nilgun Yildirim [2], Firdevs Gurer [3], Hikmet Basmak [2], Kubilay Uzuner [1], Varol Sahinturk [3], Huseyin Gursoy [2]

[1]Department of Physiology, Eskisehir Osmangazi University Medical Faculty, Eskisehir 26480, Turkey
[2]Department of Ophthalmology, Eskisehir Osmangazi University Medical Faculty, Eskisehir 26480, Turkey
[3]Department of Histology, Eskisehir Osmangazi University Medical Faculty, Eskisehir 26480, Turkey
Correspondence to: Yildirim Nilgun. Department of Ophthalmology, Eskisehir Osmangazi University Medical Faculty, Eskisehir 26480, Turkey. nyyildirim@yahoo.com
Received: 2015-03-07 Accepted: 2015-04-29

Abstract

• AIM: To study the effect of vasoactive intestinal peptide (VIP) on wound healing in experimental alkali burns of the cornea.

• METHODS: Twenty-seven albino rabbits, weighing 3.2±0.75 kg were used. Alkali burns were induced on corneas by applying 10 mm Whatman paper No:50 soaked in 1 mol/L NaOH. They have further classified into 5 groups as follows: 1) control group given no treatment (n=5); 2) VIP given subconjunctivally (n=6); 3) VIP injected into anterior chamber (n=6); 4) NaCl 0.9% given subconjunctivally (n=5); 5) NaCl 0.9% given into the anterior chamber (n=5). All treatment protocols except control group were followed by topical eye drops composed of VIP at two hourly intervals for one week from 8 a.m. to 6 p.m.

• RESULTS: VIP treated groups of rabbits with alkali burns were found to have better wound healing findings histo-pathologically when compared to those of control group who have received no treatment on day 30. No differences were observed between groups in respect to degree of polymorphonuclear leukocytes (PMNL) infiltration and degree of loss of amorphous substrate on day 15. However, PMNL infiltration and degree of loss of amorphous substrate were lower in Groups 2 and 3 when compared to that of control group on day 30 (P<0.05).

• CONCLUSION: We have shown that VIP has positive effects on alkali induced corneal burns. VIP may inhibit PMNL migration to cornea through an immunomodulatory effect. Inhibition of PMNL migration might reduce the release of collagenases and this might prevent the extracellular amorphous substance loss.

• **KEYWORDS:** vasoactive intestinal peptide; alkali-burned cornea; wound healing; alkali burn; rabbit
DOI:10.18240/ijo.2016.02.04

Tuncel N, Yildirim N, Gurer F, Basmak H, Uzuner K, Sahinturk V, Gursoy H. Effect of vasoactive intestinal peptide on the wound healing of alkali-burned corneas. *Int J Ophthalmol* 2016;9(2):204-210

INTRODUCTION

Alkali burn is one of the most severe corneal injuries. The alkali-burned cornea frequently leads to blindness, scar tissue formation or ulceration. The treatment and complications of alkali-burned corneas are one of the most important and challenging problems in ophthalmology [1-3]. Several studies have focused on the patho-physiological changes, on the effects of various agents, and on the treatment methods of wound healing in alkali-burned corneas[3-5].

It has been emphasized that polymorphonuclear leukocytes (PMNL) have a major role in a series of complex events taking place in the corneal tissue after alkali burns [6-7]. It is possible that these inflammatory cells release chemotactic agents that recruit more PMNLs [8]. This PMNL infiltration may induce the corneal cells in healthy portions of the injured corneas to synthesize polypeptides such as IL-6, IL-8, IL-12 and PDGF *etc* that are chemo attractant to inflammatory cells [8-9]. Therefore, agents that block PMNL migration and/or inactive mediators excreted by them may be tried in the treatment of alkali-burned corneas.

Experimental and clinical studies reported various treatment modalities which were effective in early and late phase wound healing and were successful in reducing the risk of corneal perforation. These treatment modalities were as follows: 1) agents that prevent the migration of PMNLs and respiratory burst, such as sodium citrate[10-12]; 2) cyanoacrylate glued-on contact lenses that block the corneal infiltration of PMNLs mechanically [13]; 3) collagenase inhibitors like acetyl-cystein and tetracycline [14-15]; 4) anti-inflammatory agents like corticosteroids and medroxy progesterone acetate[16-17]; 5) agent that increase collagen synthesis, such as ascorbic acid [11,18]. Other than those state above, agents facilitating epithelization and surgical methods are also being used in the treatment[19-21].

Vasoactive intestinal peptide (VIP) is a 28 amino acid neurotransmitter peptide that is widely distributed particularly in the central and peripheral nervous system [22]. VIP has cytoprotective effects in many tissues and organs of the body particularly in the lung injury [23-25]. A number of mechanisms may explain the ability of VIP to protect against tissue damage. These include its modulatory effects on inflammatory cell function and its anti-oxidant capacity[26-28]. However, there is very little data in the literature regarding the protective effect of VIP in alkali-burned tissues.

In this study, we investigated the clinical and histological effects of VIP applied locally in the early phase wound healing of the alkali-burned corneas in rabbits.

MATERIALS AND METHODS

The study was approved by the Institutional Animal Care and Use Committee of Eskisehir Osmangazi University Medical Faculty (EOUMF). All of the experimental procedures were performed at the EOUMF physiology department according to the guiding principles for the care and use of animals (Anadolu University Animal Experiments Local Ethics Committee Guidelines). The histo-pathological evaluation was performed by the EOUMF histology department.

Twenty-seven albino rabbits of either sex, weighing 3.0-3.5 kg were used. Same conditions were provided for all rabbits through the experiment. Local anesthesia was conducted by using oxybuprocaine 0.5% topically on the right eyes before the experiment three times at five-minute intervals. After retracting the lids with a speculum alkali burns were induced by keeping round pieces of Whatman paper No:50 with a diameter of 10 mm soaked in 1 mol/L NaOH on the surface of corneas for seconds after which the cornea and inner aspects of the eyelids were irrigated with NaCl 0.9% for 1min.

Preparation of Vasoactive Intestinal Peptide 1) Stock solution: by solving VIP (V3628) provided from the Sigma Company in 1 mL of cold NaCl 0.9%, stock solution with a concentration of 5 mg/mL was prepared; 2) VIP solution for anterior chamber injections: stock solution (5 mg/mL) was used for anterior chamber in a concentration of 5 ng/mL; 3) VIP solution for subconjunctival injection: stock solution of VIP (5 mg/mL) was diluted in 0.9% cold NaCl to yield a final concentration of 250 ng/mL. Out of which 0.2 mL was used for subconjunctival injection; 4) VIP drop solution: to get 25 mg per drop (50 mL), stock solution was diluted in 0.9% cold NaCl to yield a final concentration of 0.5 mg/mL. All diluted and stock solutions (pH 7.4) were divided as single doses in polyethylene tubes and kept in -85℃ and in room temperature before use.

Treatment Protocol All treatment protocols except control group were followed by topical eye drops composed of VIP. One drop of VIP solution was instilled at two hourly intervals for one week in all groups except the control group. Rabbits after inducing alkali burn were treated as follows: Group 1 (n=5): controls, no treatment was given after inducing burn; Group 2 (n =6): after inducing burn, a single dose of 1 mL solution of VIP (5 ng/mL) was injected into the anterior chamber with Hamilton injector followed by topical eye drops (25 ng/50 mL) at two hourly intervals for one week from 8 a.m. to 6 p.m; Group 3 (n =6): after inducing burn, a single dose of VIP solution (50 ng/0.2 mL) was injected subconjunctivally. Treatment protocol was continued by topical eye drops (50 mL=25 ng) at two hourly intervals for one week from 8 a.m. to 6 p.m; Group 4 (n=5): 1 mL of NaCl 0.9% was injected into the anterior chamber with a Hamilton injector followed by topical eye drops (25 ng/50 mL) at two hourly intervals for one week from 8 a.m. to 6 p.m; Group 5 (n =5): NaCl 0.9% was injected subconjunctivally after inducing burn and topical eye drops were continued at two hourly intervals for one week from 8 a.m. to 6 p.m.

Clinical Observation and Histo-pathological Examination

All rabbits underwent bio-microscopical examinations on day 1, 3, 5, 7, and 15 after inducing alkali burn. Their photographs were taken using a Canon 100 macro camera. The final visit was on day 15 for twelve rabbits, while it was on day 30 for the others. Corneal epithelization and depth of ulceration were evaluated clinically (Figure 1). The percentage corneal epithelization was evaluated after staining with 0.5% methylene blue dye. The size of the region with epithelial defect taking the dye was measured on the photographs. The corneal ulcers were categorized according to the depth of the lesion[18]. Stage 0: no ulcer (intact cornea); stage 1: superficial; stage 2: medium-depth; stage 3: deep; stage 4: desmatocele and stage 5: corneal perforation. On day 15, three rabbits died, corneal perforation developed in four rabbits and keratitis developed in five rabbits. Therefore, corneal buttons were obtained from 12 rabbits on day 15 following alkali-burn (n=2, Group 1; n=3, Group 2; n=3, Group 3; n=2, Group 4; n=2, Group 5). The other corneal buttons were obtained on day 30 following alkali-burn. For light microscopy corneas were fixed in 4% formaldehyde, and dehydrated in ethanol before embedding in paraffine. The center, two other points within 6 mm of the central cornea and a point from the peripheral cornea were examined. Of 4 μm thick antero-posterior conventional sections were taken from the paraffine-embedded corneal buttons. Ten sections were cut and five individual measurements were made on each section by two examiners (Gurer F, Sahinturk V). The average grades were documented for each animal. After staining with hematoxylin-eosin, central and peripheral corneal epithelium, stroma and endothelium were examined under light microscope (Figures 2, 3). In addition, toluidine blue 1% aqueous solution was used to examine the structure of glycose amino-glycans (GAGs) (Figure 4).

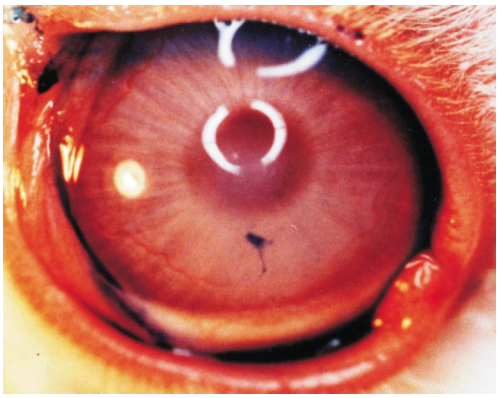


Figure 1 Macroscopically intact cornea after applying methylene blue on day 30 in one of Group 2 rabbits (after the injection of VIP into the anterior chamber and topical application of VIP).

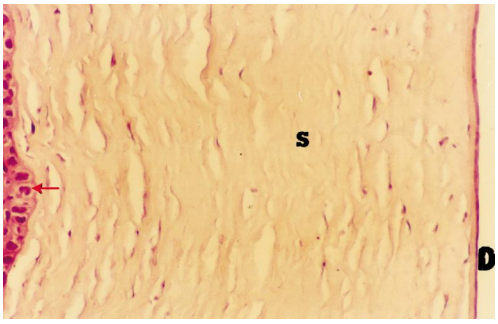


Figure 2 Reepithelization in the alkali burned cornea in which VIP was injected into the anterior chamber (→); Near to normal edematous stroma and cornea with no PMNL infiltration (S). Descemet´s membrane and endothelium (D). HE×100.

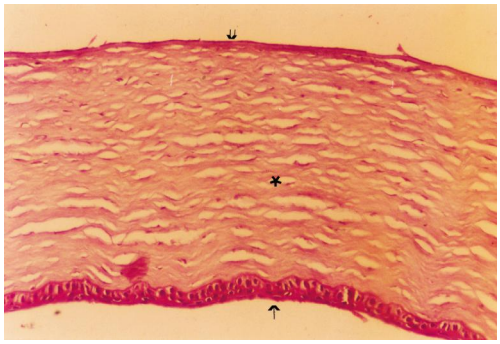


Figure 3 Alkali burned cornea in a control rabbit: anterior and midstroma are infiltrated with PMNLs (*); Posterior stroma is near to normal. Posterior stroma (⇒); Anterior stroma (→). HE×20.

In the histological examination, the presence of the epithelial layer, its structure, its connection to the Bowman's membrane and the presence of intraepithelial PMNL infiltration were evaluated. Anterior and posterior stroma were evaluated for the regular, arrangement of collagen, fiber, bundles or loss of collagen fibers, the status of keratocytes and amorphous substance and the density of PMNL infiltration. PMNL density was examined in both peripheral and central cornea. As stromal amorphous substance has a metachromatic feature, in preparations stained with toluidine blue, loss of

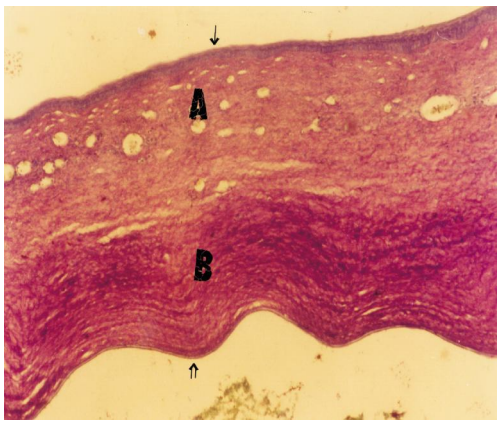


Figure 4 GAG loss in the involved areas of alkali burned cornea(A) in one of the control rabbits; Deep stroma with GAGs protected and collagen bundles regularly arranged (B). Epithelium (→); Descemet´s membrane (⇒). Toluidine Blue×20.

stromal collagenous ground substance was investigated and grading was made as follows: loss in the anterior stromal region: grade l; loss in the middle: grade 2 and in the posterior: grade 3. Similar grading was made for PMNLs also. Infiltration only at the level of anterior stromal region: grade 1, till the level of middle stroma: grade 2 and infiltration of all stroma: grade 3. The clinical and histological findings at the final visit were analyzed. The histological findings on day 15 and 30 were compared between groups separately. However, the clinical findings at the final visit, which was on day 15 for 12 rabbits and on day 30 for 15 rabbits, were compared between groups as a whole.

Statistical Analysis The non-parametric Kruskal-Wallis one-way analysis of variance by ranks and Tukey's HSD multiple comparisons tests were used for statistical analysis. $P<0.05$ were required for statistical significance. Statistical analyses were performed using IBM SPSS statistics 20.

RESULTS

The comparison of the clinical findings at the last visit (day 15 for 12 rabbits and day 30 for 15 rabbits) using the non-parametric Kruskal-Wallis one-way analysis of variance by ranks test was shown in Table 1. The mean depth of corneal lesions was mildest in Group 5 (1.60±1.52), while the mean depth was the most severe in Group 4 (3.60±1.67). The corneal epithelization observed after staining was best in Group 2 (75.00±24.29). The clinical findings among the groups were not statistically significantly different. The scores about PMNL infiltration, and loss of collagen amorphous substrate were shown in Table 2.

No differences were observed between groups in respect to degree of PMNL infiltration and loss of collagen amorphous substrate on day 15. The comparison of the histologic findings on day 15, using the non-parametric Kruskal-Wallis one-way analysis of variance by ranks test was shown in Table 3. However, differences were significant on day 30 ($P<0.05$). The comparison of the histologic findings on day 30, using the non-parametric Kruskal-Wallis one-way analysis

Table 1 The comparison of the clinical findings at the last visit

Clinical findings	Groups	Median values	$\bar{x} \pm s$	*P*
Depth of corneal ulceration	1	3.00 (2.00-4.00)	3 .00±1.22	
	2	2.00 (0-4.00)	2.00±1.89	
	3	1.50 (0.75-4.25)	2.17±1.94	0.354
	4	4.00 (2.00-5.00)	3.60±1.67	
	5	1.00 (0.50-3.00)	1.60±1.52	
Percentage of corneal epithelization	1	50.00 (45.00-70.00)	56.00±15.17	
	2	75.00 (50.00-100.00)	75.00±24.29	
	3	75.00 (37.50-85.00)	66.67±26.58	0.614
	4	50.00 (45.00-77.50)	59.00±18.17	
	5	60.00 (40.00-80.00)	60.00±20.00	

The 25th and 75th percentile values are given in parentheses along with the medians.

Table 2 The histological evaluation on day 15 and 30 in rabbits with alkali-burned corneas

Animals	Duration of follow-up (d)	Histological evaluation		Groups
		Depth of PMNLs infiltration	Loss of extracellular amorphous substance	
1	15	3	3	1
2	15	2	2	1
3	30	3	3	1
4	30	3	2	1
5	30	3	3	1
6	15	3	1	2
7	15	3	1	2
8	15	2	1	2
9	30	1	0	2
10	30	1	1	2
11	30	0	0	2
12	15	3	1	3
13	15	2	1	3
14	15	3	1	3
15	30	1	0	3
16	30	2	2	3
17	30	1	0	3
18	15	3	3	4
19	15	3	3	4
20	30	2	2	4
21	30	3	3	4
22	30	2	2	4
23	15	2	1	5
24	15	2	3	5
25	30	2	3	5
26	30	2	2	5
27	30	2	2	5

of variance by ranks test was shown in Table 4. Multiple comparisons by Tukey's HSD were used to analyze the results on day 30 and statistically significant comparisons were shown in Table 5. PMNL infiltration and loss of amorphous substrate were lower in Groups 2 and 3 when compared to that of control group (Group 1) on day 30. The median value for the PMNL infiltration was 1.0 (0.3-1.0) and 1.0 (1.0-1.8) in Groups 2 and 3, respectively. The median value for the PMNL infiltration was 3.0 (3.0-3.0) in Group 1. The median value for the loss of collagen amorphous substrate was 0 (0-0.8) and 0 (0-1.5) in Groups 2 and 3, respectively. The median value for the loss of collagen amorphous substrate was 3.0 (2.3-3.0) in Group 1.

DISCUSSION

In the current study, local application of VIP was tried for the therapy of alkali-burned corneas in rabbits. The clinical findings namely, the depth of corneal ulceration and the percentage of corneal epithelization were partially improved in corneas in which the VIP was injected into the anterior chamber (Group 2) or under the conjunctiva (Group 3) in addition to the topical instillation of VIP drops. However, the difference failed to reach a statistical significance. The histological findings were also better in Groups 2 and 3 on day 30. The difference was statistically significant.

The principal goal of therapy in alkali induced corneal burn is the prevention of corneal ulceration and perforation[2-3]. The appropriate treatment of severe burns still continues to be a difficult and complex problem. Several treatment modalities have been used to treat severe alkali-burned corneas. Up to date, no single treatment was found to be effective during the wound healing process after alkali burns [2-3]. In wound healing, new tissue formation starts with inflammation and re-epithelization [29]. These are driven in part by a complex mixture of growth factors, cytokines, and neuro-peptides which are released coordinately into the area of injury [30-31]. VIP has been suggested to play a role in local inflammatory processes by suppression of chemotaxis and MMP expression elicited by some cytokines and chemokines [32]. In this study, we used VIP, which is an anti-inflammatory agent and an immunomodulator in the prevention of tissue damage, in the experimental alkali-burned rabbit cornea model[26].

The clinical and histological findings in alkali burned corneas on day 15 were similar. On day 30, especially corneas treated with VIP showed better results in terms of density of PMNLs and protection of extracellular amorphous substance. These results suggest that VIP may be beneficial in alkali burned corneal wound healing. To the best of our knowledge, this is the first report about the effect of VIP on alkali burns of cornea during the early phase of wound healing.

This effect of VIP may probably be explained through multiple mechanisms. PMNLs are the most important cells in the physio-pathological events that take place in the alkali-burned cornea [6,10,16]. The reasons for these cells to infiltrate into the corneal tissue are the chemotactic ability of the corneal epithelium and the alkali damaged corneal tissue itself. Alkali degraded corneal collagen has been shown in a number of studies to have a chemotactic effect for PMNLs *in vitro* [33-34]. PMNLs infiltrating into the corneal tissue cause tissue damage by inducing respiratory burst[34]. The existence of PMNLs in the cornea is thought to cause delay in the corneal wound healing for a long time [35]. Therefore, the prevention of corneal migration of PMNLs may be a major step forwards in the treatment of the alkali burned cornea. In the current study, PMNLs were less dense in corneas treated

Table 3 The comparison of the histologic findings on day 15

Histological findings	Group1(*n*=2)	Group 2 (*n*=3)	Group 3 (*n*=3)	Group 4 (*n*=2)	Group 5 (*n*=2)	*P*
Depth of PMNLs infiltration	2.0 (2.0-3.0)	3.0 (2.3-3.0)	3.0 (2.3-3.0)	3.0 (3.0-3.0)	2.0 (2.0-2.0)	0.395
Loss of extracellular amorphous substance	2.5 (2.0-3.0)	1.0 (1.0-1.0)	1.0 (1.0-1.0)	3.0 (3.0-3.0)	2.0 (1.0-3.0)	0.073

The 25th and 75th percentile values are given in parentheses along with the medians.

Table 4 The comparison of the histologic findings on day 30

Histological findings	Group 1 (*n*=3)	Group 2 (*n*=3)	Group 3 (*n*=3)	Group 4 (*n*=3)	Group 5 (*n*=3)	*P*
Depth of PMNLs infiltration	3.0 (3.0-3.0)	1.0 (0.3-1.0)	1.0 (1.0-1.8)	2.0 (2.0-2.8)	2.0 (2.0-2.0)	0.018[1]
Loss of extracellular amorphous substance	3.0 (2.3-3.0)	0 (0-0.8)	0 (0-1.5)	2.0 (2.0-2.8)	2.0 (2.0-2.8)	0.046[1]

The 25th and 75th percentile values are given in parentheses along with the medians. [1]Significant.

Table 5 The multiple comparisons of the histological findings on day 30 by Tukey's HSD

Histological findings	Compared groups	*P*
Depth of PMNLs infiltration	Group 1 *vs* 2	0.002[a]
	Group 1 *vs* 3	0.016[a]
	Group 2 *vs* 4	0.031[a]
Loss of extracellular amorphous substance	Group 1 *vs* 2	0.014[a]
	Group 1 *vs* 3	0.034[a]
	Group 2 *vs* 4	0.049[a]
	Group 2 *vs* 5	0.049[a]

[a]Significant. Group 2 showed lower PMNL infiltration when compared to that of Group 4 (*P*=0.031). Group 2 also showed less amorphous substrate loss when compared to that of Groups 4 and 5 (*P*=0.049).

with VIP. Our histological findings reveal that VIP prevents the loss of extracellular amorphous substance which confirms the above stated VIP effect. The mechanism by which VIP exerts this effect may be that VIP may protect the structure of degraded collagen which has a chemotactic effect for PMNLs [36]. Collagen fiber bundles covered by extracellular amorphous substance neither permit PMNL migration nor are they affected by collagenolytic enzymes. This appearance suggests an indirect role of VIP in the prevention of PMNL migration.

VIP is reported to have an anti-inflammatory effect which is realized through its inhibition of inflammatory cell functions (respiratory burst), blocking the cytokines' effects and/or scavenging the free oxygen radicals in the environment[26-28]. These in turn prevent tissue damage. In a report by Grimm *et al* [37], VIP acted as a potent anti-inflammatory agent by inhibiting leukocyte migrating through suppression of the function of chemokine receptors *in vivo*. It is known that the earlier the alkali induced tissue damage is prevented, the better the wound healing will be [38]. It seems that VIP affects the wound healing positively in the late phase by acting a preventive role at the beginning of this series of complex events. Better healing seen with VIP injections into the anterior chamber may be explained by the qualitative and quantitative changes taking place in the aqueous humour. Studies show an increase in aqueous humour production through cAMP by stimulation of VIP receptors on the ciliary body [39-40]. It is not known yet whether VIP has any effect on the content of aqueous humour.

One of the important problems faced in wound healing after alkali eye injuries is the impairment of tear structure which can be explained by the numerical loss of goblet cells and the obstruction of channels responsible for aqueous secretion. VIP used in this study may thus have played a regulatory role in tear structure through its receptors in conjunctiva and on goblet cells[41-43].

The strengths of the current study are the strong hypothesis based on the VIP possible effects on wound healing and the fact that this study was the first experimental trial of the use of local VIP for the treatment of alkali-burned corneas. Although several studies have been conducted to assess the effects of VIP treatment in Pseudomonas aeruginosa-infected cornea [44-47], the application of topical VIP in alkali-burned corneas have not been tested. Anti-inflammatory and also pro-inflammatory actions under appropriate conditions through cytokines and enhanced expression of growth factors have been presented in these laboratory studies. Less destruction was obtained in corneas treated with VIP. However, there are some limitations including the non-uniform severity of the alkali burns, subjective evaluation of the clinical and histological findings and the size of the study group. The clinical findings on day 15 and 30 were considered between groups as a whole, whereas the histological findings on day 15 and 30 were compared separately considering the expected changes in the inflammatory process after the induction of the alkali burns. It could be more scientific to compare all findings on day 30, but twelve of the rabbits were sacrificed on day 15.

In conclusion, we have shown that VIP has positive effects on alkali induced corneal burns. The current study may be a pioneer study for the trial of local VIP in alkali-burned corneas. Several hypotheses can be used to explain this positive effect on corneal wound healing. VIP may inhibit PMNL migration to cornea through an immunomodulatory effect. Inhibition of PMNL migration might reduce the release of collagenases and this might prevent the extracellular amorphous substance loss. In addition, VIP

might play a role by regulating the structure of the aqueous humour and tear structure. Further studies are needed to show the possible benefits of VIP in corneal wound healing following alkali burns.

ACKNOWLEDGEMENTS

Conflicts of Interest: Tuncel N, None; **Yildirim N,** None; **Gurer F,** None; **Basmak H,** None; **Uzuner K,** None; **Sahinturk V,** None; **Gursoy H,** None.

REFERENCES

1 Hamill CE, Bozorg S, Peggy Chang HY, Lee H, Sayegh RR, Shukla AN, Chodosh J. Corneal alkali burns: a review of the literature and proposed protocol for evaluation and treatment. *Int Ophthalmol Clin* 2013;53(4):185-194.

2 Gicquel JJ. Management of ocular surface chemical burns. *Br J Ophthalmol* 2011;95(2):159-161.

3 Fish R, Davidson RS. Management of ocular thermal and chemical injuries, including amniotic membrane therapy. *Curr Opin Ophthalmol* 2010;21(4):317-321.

4 Hosseini H, Nejabat M, Mehryar M, Yazdchi T, Sedaghat A, Noori F. Bevacizumab inhibits corneal neovascularization in an alkali burn induced model of corneal angiogenesis. *Clin Experiment Ophthalmol* 2007;35(8):745-748.

5 Zhao B, Ma A, Martin FL, Fullwood NJ. An investigation into corneal alkali burns using an organ culture model. *Cornea* 2009;28(5):541-546.

6 Burns FR, Gray RD, Paterson CA. Inhibition of alkali-induced corneal ulceration and perforation by a thiol peptide. *Invest Ophthalmol Vis Sci* 1990;31(1):107-114.

7 Yan H, Qi C, Ling S, Li W, Liang L. Lymphatic vessels correlate closely with inflammation index in alkali burned cornea. *Curr Eye Res* 2010;35(8):685-697.

8 Elgebaly SA, Downes RT, Bohr M, Forouhar F, O'Rourke J, Kreutzer DL. Inflammatory mediators in alkali-burned corneas: preliminary characterization. *Curr Eye Res* 1987;6(11):1263-1274.

9 Planck SR, Rich LF, Ansel JC, Huang XN, Rosenbaum JT. Trauma and alkali burns induce distinct patterns of cytokine gene expression in the rat cornea. *Ocul Immunol Inflamm* 1997;5(2):95-100.

10 Paterson CA, Williams RN, Parker AV. Characteristics of polymorphonuclear leucocyte infiltration into the alkali burned eye and the influence of sodium citrate. *Exp Eye Res* 1984;39(6):701-708.

11 Pfister RR, Haddox JL, Yuille-Barr D The combined effect of citrate/ascorbate treatment in alkali-injured rabbit eyes. *Cornea* 1991;10(2):100-104.

12 Pfister RR, Haddox JL, Snyder TL. Topical citrate inhibits the adherence of neutrophils to postcapillary venules. *Cornea* 1990;9(3):238-245.

13 Spelsberg H, Sundmacher R. Significance of immediate affixation of a hard contact lens in the emergency treatment of severe alkali burns of the cornea (case report). *Klin Mondbl Augenheilkd* 2005;222(11):905-909.

14 Schultz GS, Strelow S, Stern GA, Chegini N, Grant MB, Galardy RE, Grobelny D, Rowsey JJ, Stonecipher K, Parmley V. Treatment of alkali-injured rabbit corneas with a synthetic inhibitor of matrix metalloproteinases. *Invest Ophthalmol Vis Sci* 1992;33(12):3325-3331.

15 Chandler HL, Gemensky-Metzler AJ, Bras ID, Robbin-Webb TE, Saville WJ, Colitz CM. In vivo effects of adjunctive tetracycline treatment on refractory corneal ulcers in dogs. *J Am Vet Med Assoc* 2010;237(4):378-386.

16 Foster CS, Zelt RP, Mai-Phan T, Kenyon KR. Immunosuppression and selective inflammatory cell depletion. Studies on a guinea pig model of corneal ulceration after ocular alkali burning. *Arch Ophthalmol* 1982;100(11):1820-1824.

17 Crabb CV. Endocrine influences on ulceration and regeneration in the alkali-burned cornea. *Arch Ophthalmol* 1977;95(10):1866-1870.

18 Pfister RR, Paterson CA, Hayes SA. Topical ascorbate decreases the incidence of corneal ulceration after experimental alkali burns. *Invest Ophthalmol Vis Sci* 1978;17(10):1019-1024.

19 Chan CC, Biber JM, Holland EJ. The modified Cincinnati procedure: combined conjunctival limbal autografts and keratolimbal allografts for severe unilateral ocular surface failure. *Cornea* 2012;31(11):1264-1272.

20 Lim M, Goldstein MH, Tuli S, Schultz GS. Growth factor, cytokine and protease interactions during corneal wound healing. *Ocul Surf* 2003;1(2):53-65.

21 Bachmann B, Cursiefen C. Late complications after chemical burns of the ocular surface. Surgical strategies for ocular surface reconstruction. *Ophthalmologe* 2011;108(10):929-938.

22 Waschek JA. VIP and PACAP: neuropeptide modulators of CNS inflammation, injury, and repair. *Br J Pharmacol* 2013;169(3):512-523.

23 Alston EN, Parrish DC, Hasan W, Tharp K, Pahlmeyer L, Habecker BA. Cardiac ischemia-reperfusion regulates sympathetic neuropeptide expression through gp130-dependent and independent mechanisms. *Neuropeptides* 2011;45(1):33-42.

24 Uzuner K, Tunçel N, Aydin Y, Tunçel M, G ü rer F, Benli P, Ak D. The effect of vasoactive intestinal peptide (VIP) on superoxide dismutase and catalase activities in renal tissues of rats exposed to hemorrhagic ischemia reperfusion. *Peptides* 1995;16(5):911-915.

25 Wilk P. Vasoactive intestinal peptide inhibits the respiratory burst in human monocytes by cyclic AMP-mediated mechanism. *Regul Pept* 1989;25(2):187-197.

26 Delgado M, Ganea D. Vasoactive intestinal peptide: a neuropeptide with pleiotropic immune functions. *Amino Acids* 2013;45(1):25-39.

27 Vacas E, Bajo AM, Schally AV, S á nchez-Chapado M, Prieto JC, Carmena MJ. Antioxidant activity of vasoactive intestinal peptide in HK2 human renal cells. *Peptides* 2012;38(2):275-281.

28 Misra BR, Misra HP. Vasoactive intestinal peptide, a singlet oxygen quencher. *J Biol Chem* 1990;265(26):15371-15374.

29 Santoro MM, Gaudino G. Cellular and molecular facets of keratinocyte reepithelization during wound healing. *Exp Cell Res* 2005;304(1):274-286.

30 Ebihara N, Matsuda A, Nakamura S, Matsuda H, Murakami A. Role of the IL-6 classic- and trans-signaling pathways in corneal sterile inflammation and wound healing. *Invest Ophthalmol Vis Sci* 2011;52(12):8549-8557.

31 Saika S. Yin and yang in cytokine regulation of corneal wound healing: roles of TNF-alpha. *Cornea* 2007;26(9 Suppl 1):S70-74.

32 Goetzl EJ, Pankhaniya RR, Gaufo GO, Mu Y, Xia M, Sreedharan SP. Selectivity of effects of vasoactive intestinal peptide on macrophages and lymphocytes in compartmental immune responses. *Ann N Y Acad Sci* 1998;840:540-550.

33 Pfister RR, Haddox JL, Sommers CI. Alkali-degraded cornea generates a low molecular weight chemoattractant for polymorphonuclear leukocytes. *Invest Ophthalmol Vis Sci* 1993;34(7):2297-2304.

34 Pfister RR, Haddox JL, Lam KW. Alkali-degraded cells generate a respiratory burst stimulant for neutrophils. *Cornea* 1993;12(2):155-160.

35 Zhang Y, Kobayashi T, Hayashi Y, Yoshioka R, Shiraishi A, Shirasawa S, Higashiyama S, Ohashi Y. Important role of epiregulin in inflammatory responses during corneal epithelial wound healing. *Invest Ophthalmol Vis Sci* 2012;53(4):2414-2423.

36 Pfister RR, Haddox JL. A neutrophil chemoattractant is released from cellular and extracellular components of the alkali-degraded cornea and blood. *Invest Ophthalmol Vis Sci* 1996;37(1):230-237.

37 Grimm MC, Newman R, Hassim Z, Cuan N, Connor SJ, Le Y, Wang JM, Oppenheim JJ, Lloyd AR. Cutting edge: vasoactive intestinal peptide acts as a potent suppressor of inflammation in vivo by trans-deactivating chemokine receptors. *J Immunol* 2003;171(10):4990-4994.

38 Brodovsky SC, McCarty CA, Snibson G, Loughnan M, Sullivan L, Daniell M, Taylor HR. Management of alkali burns: an 11-year retrospective review. *Ophthalmology* 2000;107(10):1829-1835.

39 Mittag TW, Tormay A, Podos SM. Vasoactive intestinal peptide and intraocular pressure: adenylyl cyclase activation and bindings sites for vasoactive intestinal peptide in membranes of ocular ciliary processes. *J Pharmacol Exp Ther* 1987;241(1):230-235.

40 Koh SW, Yue BY. VIP stimulation of cAMP production in corneal endothelial cells in tissue and organ cultures. *Cornea* 2002;21 (3): 270-274.

41 Cox SM, Nichols JJ. The neurobiology of the meibomian glands. *Ocul Surf* 2014;12(3):167-177.

42 Dartt DA. Signal transduction and control of lacrimal gland protein secretion: a review. *Curr Eye Res* 1989;8(6):619-636.

43 Li D, Jiao J, Shatos MA, Hodges RR, Dartt DA. Effect of VIP on intracellular [Ca2+], extracellular regulated kinase 1/2, and secretion in cultured rat conjunctival goblet cells. *Invest Ophthalmol Vis Sci* 2013;54 (4):2872-2884.

44 McClellan S, Jiang X, Barrett R, Hazlett LD High-mobility group box 1: a novel target for treatment of Pseudomonas aeruginosa keratitis. *J Immunol* 2015;194(4):1776-1787.

45 Berger EA, Vistisen KS, Barrett RP, Hazlett LD. Effects of VIP on corneal reconstitution and homeostasis following Pseudomonas aeruginosa induced keratitis. *Invest Ophthalmol Vis Sci* 2012;53(12):7432-7439.

46 Jiang X, McClellan SA, Barrett RP, Zhang Y, Hazlett LD. Vasoactive intestinal peptide downregulates proinflammatory TLRs while upregulating anti-inflammatory TLRs in the infected cornea. *J Immunol* 2012;189(1): 269-278.

47 Jiang X, McClellan SA, Barrett RP, Berger EA, Zhang Y, Hazlett LD. VIP and growth factors in the infected cornea. *Invest Ophthalmol Vis Sci* 2011;52(9):6154-6161.