ARAŞTIRMA

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Effect of Autologous Serum on Healing of Corneal Endothelium in Experimentally-Induced Alkaline Burns of The Cornea in Rabbits*

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In this study, the effectiveness of autologous serum on corneal endothelium in alkaline burns of the cornea was investigated.

Alkaline burns lead to severe trauma on the cornea. Cornea healing is quite difficult and complicated. Endothelial layer is responsible for preserving transparency of the cornea. Repair of the cell injury in the endothelial layer is achieved through migration of healthy cells to the injured area. Autologous serum reduces injury of the endothelial layer through inhibiting collagenase activity and accelerating cell migration.

In the study, a total of 21 New Zealand rabbits were divided into 3 groups, 7 in each group. Alkaline burn was created in the right eyes with 2N NaOH in groups II and III under general anesthesia and saline solution was administered in group II and autologous serum was administered in group III four times a day for 21 days. In group I, autologous serum was administered four times a day for 21 days without creating alkaline burn. At the end of 21th day, corneas of the decapitated rabbits were removed and histopathologically examined. Endothelial defect areas and endothelial cell loss were lower in group III compared to group II. In this study, it is suggested that the use of autologous serum was seen to be effective on the healing of the endothelium in the alkaline induced corneal burns.

Anahtar Kelimeler: *Cornea, endothelium, alkaline burn, autologous serum.*

Tavşanlarda Deneysel Oluşturulan Kornea Alkali Yanıklarında Otojen Serumun Kornea Endotel İyileşmesi Üzerine Etkisi

Bu çalışmada korneanın alkali yanıklarında otolog serumun kornea endoteli üzerindeki etkinliği araştırılmıştır.

Alkali yanıklar korneada çok şiddetli travmalar oluştururlar. Bu nedenle iyileşmesi oldukça zor ve komplikasyonlu olur. Endotel tabaka korneanın şeffaflığının korumasında görevlidir. Endotel tabakada oluşacak hücre hasarının onarılması çevredeki sağlam hücrelerin hasarlı alana migrasyonu ile olmaktadır. Otolog serum kologenaz aktivitesini inhibe ederek endotel tabakasındaki hasarı azaltır ve hücrelerin migrasyonunu hızlandırır.

Çalışmada 3 grupta toplam 21 adet Yeni Zelanda tavşanı kullanıldı. Genel anestezi altında II. ve III. gruplarda 2 N NaOH ile sağ gözlerde korneada alkali yanık oluşturuldu ve 21 gün süreyle II. grupta serum fizyolojik, III. grupta ise otojen serum günde 4 kez uygulandı. I. grupta ise alkali yanık oluşturulmadan 21 gün süreyle günde 4 kez otojen serum uygulandı. Yirmibirinci günün sonunda ötenazi edilen tavşanların korneaları çıkartılarak histopatolojik muayeneleri yapıldı. III. gruptaki endotel defekt alanları ve endotel hücre kaybının II. gruba göre daha az olduğu tespit edildi. Bu çalışmada otojen serumun kornea alkali yanıklarında endotel iyileşmesi üzerine yararlı etkisinin olduğu görüldü.

Key Words: *Kornea, endotel, alkali yanık, otojen serum.*

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Introduction

Severe ocular injury occurs in alkaline burns of the cornea. Alkalines cause more severe destruction in the cornea and conjunctiva than acids as they denaturate corneal collagenes by rapidly penetrating into tissues. Lesions develop mainly in corneal and conjunctival epithelium and stroma, endothelium, episclera depending on the degree of penetration of alkaline substance (1-4).

Endothelial layer of the cornea is a thin layer composed of single layer cells covering the innermost surface of the cornea. Endothelium serves as a barrier against diffusion of aqueous humor into stroma. It also pumps out the accumulated water in the stroma. By this means, collagenous fibers of the stroma forms a regular structure and helps

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maintenance of transparency of the cornea (5-8). Aging, surgical traumas, alkaline burns, cataract extraction, lens implantation, penetrating keratoplasty and traumas induced by other intraocular surgeries lead to injury in endothelium layer (9-11). Endothelial cells of the cornea occur rarely in vivo mitosis in order to compensate cell death and cell loss. Endothelial losses are compensated by filling the lost areas are by forming conjugation via neighboring cells (12-14).

Autologous serum is used in various ocular surface diseases like persistent epithelium defect and keratoconjunctivitis sicca (15-17). Biochemical and biomechanic contents of autologous serum are similar to those of normal lacrima. Growth factors in the serum aid healing of epithelial defects and other layers of the cornea by inhibiting collagenase activity (18-20).

The aim of the present study was to investigate the effects of autologus serum with anticollagenase effect on endothelial cell count and morphology in the alkaline induced corneal burns.

Materials and Methods

Animals and experimental design: A total of 21 healthy New Zealand Rabbits weighing 2.5-3 kg were used in the study. Rabbits were kept in special cages and maintained during the study period in Experimental Research Center of Fırat University. Animals were randomly divided into 3 groups 7 in each group. Group I was control group (autologous serum), group II was alkaline burn+saline solution (AB+SS) group, group III was alkaline burn + autogenous serum (AB+AS) group.

Anesthesia technique: Rabbits in group II and III were anaesthetised for the experimental procedure, the rabbits were firstly injected with i.m. 5 mg/kg xylazine hydrochloride (Rompun, Bayer, İstanbul, Turkey) followed by 35 mg/kg ketamine hydrochloride (Ketalar, Eczacıbaşı, İstanbul, Turkey). Analgesia was provided by dropping 0.5% proparacaine hydrochloride (Alcaine ophtalmic solution 0.5%, Alcon Laboratories, Istanbul, Turkey) on the cornea before creating burn.

Creating alkaline burn: After right eyes of the rabbits in groups II and III under general anesthesia were opened with a Blepharostat , a filter paper with 6 mm in diameter was immersed into 2 N NaOH and placed in the center of the cornea and waited for 1 minute. The cornea was then washed with saline solution for 2 minutes.

Preparation of autologous serum and administration: After shaving of the outer surface of ear, 8-10 mL of blood was drawn from vena auricularis with a vacutainer. The blood samples were centrifuged at 4000 rpm for 10 min to obtain autologous serum. Obtained serum was stored at +4 ºC until use.

Three drops of non-diluted autologous serum were dropped in the eyes of rabbits in group I.

Three drops of saline were dropped in the eyes of rabbits with corneal burn in group II

Three drops of non-diluted autologous serum were dropped in the eyes of rabbits with corneal burn in group III. All administrations were made 4 times a day. Applications were continued for 21 days in all three groups

Histopathological examination: Rabbits were decapitated at the end of 21th day. Removed corneas were washed twice with phosphate-buffer saline (PBS). Afterwards, they were fixed with 3.7% paraformaldehyde-PBS solution at room temperature for 10 min. Endothelium layer of the corneas were washed with PBS again, and they were removed together with descement membrane using microscope knife under Stereo microcscope (Olympus SZX16). Removed layer was washed again twice with PBS and then placed on the microscope slide. It was held for 3 min at -20 ºC acetone slides and washed with PBS several times, and were incubated with 1% mixture of bovine serum albumin (BSA)-PBS for 10 min before fluorescence staining. *N*-(7-Nitrobenzofurazan-4-yl) phallacidine (Sigma - Cas No:73413-78-2) was used for fluorescence staining. Staining was carried out according to manufacturer instructions. Methanolic phallicidine solution was obtained by adding 1.5 mL methanol on 300 U phallacidine. Each slide was incubated with 5 µL methanolic phallacidine + 200 µL PBS solution at room temperature. Slides were then examined under fluorescence microscope (Olympus BX51) (x40). Seven fields were randomly chosen from each slide and they were examined. Defective areas were classified as severe +++ (7 or more), moderate++ (between 4-6) and mild+ (between 1-3 defect areas).

Horizontal and vertical lengths of the defective areas in groups II and III were measured morphometrically. Similarly, endothelial cells in 1 mm^2 of each slide in all three groups were counted (8, 13).

Statistical analysis: The data are expressed as mean±SEM. $P<0.05$ value was considered to be significant. Vertical and horizontal defective areas in groups II and III were compared by sample t-test. The differences in endothelial cells in all three groups were analysed by one-way analysis of variance (ANOVA) and post hoc. Tukey test SPSS statistical program (version 15.0) was used for the analysis of data

Results

A normal endothelium layer was seen in rabbits in group I (Figure 1). While severe defective areas were detected in 4 rabbits in group II, moderate defective areas were detected in 2 and mild defective areas were detected in 1 (Figure 2); moderate defective areas were detected in 2 rabbits in group III and mild defective areas were detected in 5 rabbits (Figure 3). Regenerated cells at borders of defective area were polygonal and consisted of f-actin microspikes. Microspikes in groups II and III were seen to be consistent with the defects. Quantitative but non-morphometric differences were seen in defective areas between the groups (Table 1). Differences were also detected in cell intensities in

endothelium layer. An average of 2797 cells/mm^2 were counted in group I, 2001 cells/mm^2 in group II and 2625 cells/mm^2 in group III (Table 2). The use of autologous serum significantly (P<0.05) improved the decreases in cell count induced by alkaline burn.

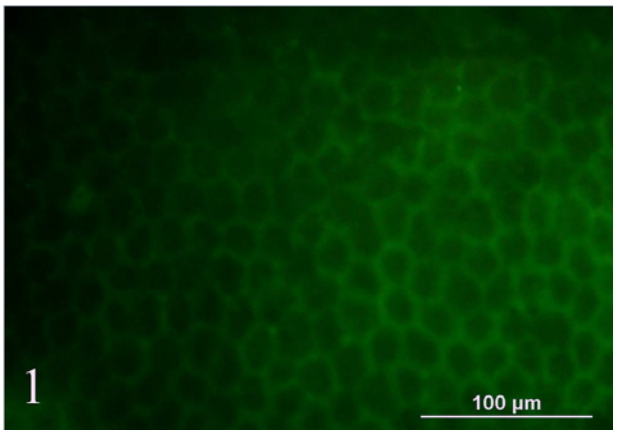

Figure 1. Microscopic appearance of normal endothelium layer in group I x40

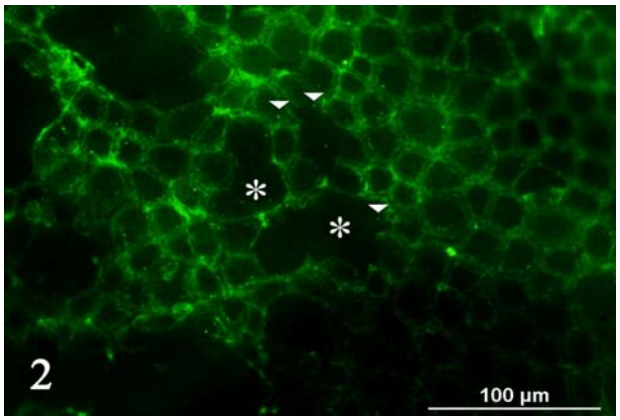

Figure 2. Severe defect in group II (*) and f-actin microspikes (arrow heads)x40

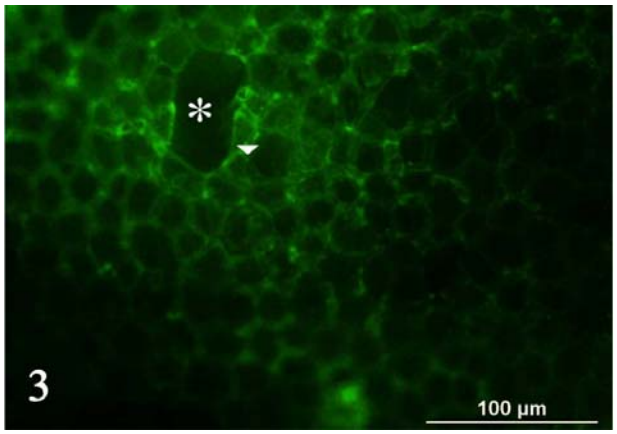

Figure 3: Mild defect in group III (*) and f-actin microspikes (arrow head)x40

Table 1. Morphometric measurument of defective areas in groups II and III

Variables	Group II (n: 7)	Group III (n: 7)	Significance
Vertical lenght	51.42±5.20	44.28±4.68	NS
Horizontal lenght	59.28±6.30	50.00±4.22	NS

NS: Not significant

Table 2. Mean cell counts per 1 mm^2 endothelium areas in I, II and III groups

Groups	n	Mean±SE
I	7	2797.14±17.55^a
II	7	2001.42±12.42^b
III	7	2625.71±21.02^c

^{a,b,c}: Different superscript letters in each column show significant differences (P<0.05)

Discussion

A transparent cornea is an indicator for a healthy endothelial layer. Endothelial loss may occur due to various reasons. If there is much loss, the cornea cannot keep normal water proportion, it swells out and losses its refractive ability. In this study, it was aimed to determine the effectiveness of autologous serum in the prevention of endothelial injury in experimentally-induced alkaline burns.

Endothelial cells have no regeneration ability and mitotic activity (11, 14). Neighboring endothelial cells expand and migrate to injury site to supply to the healing of endothelial layer. It has been reported (6, 7) that mitosis developed in endothelial cell cultures does not capable of the prevention endothelial losses. Many factors take part in endothelial healing of the cornea. Of them, presence of endothelial growth factor receptors was shown in studies. Iguchi and Komiyama (21) stated that local applications of endothelial growth factor released from lacrimal gland in cornea injuries accelerated endothelial wound healing by leading to density increase in endothelial cells and migration. Researchers (2, 4, 18) reported that epidermal growth factor (EGF) increased mitosis at the rate of 70% in alkaline burns and provided endothelial proliferation and, EGF was also effective in preserving of hexagonal shape of endothelial cells. The improvements observed in defective areas of group III when compared to group II show that autologous serum administration in corneal burns is effective for the prevention of hexagonal formations.

Autologous serum has been reported to be beneficial in alkaline induced burns, which have high collagenase activity, because it contains growth factors (15, 17, 20). Migration in endothelial cells is accomplished by f-actin molecules in cytoplasm. Meanwhile cells become longer and flat, cell count decreases and cell size increases (12, 22, 23). In the present study, presence of many f-actin molecules together with a distinctive increase in cell sizes in group II is an indicator of endothelial cell migration. In group III, the presence of normal cell sizes

the decrease in the size of defective areas and quite limited level of f-actin indicate that cell injury developes less after administration of autologous serum in this study.

Researchers (5, 24) stated that endothelial cell density should be between 2500-3000 cells/mm^2 in a healthy individual. Aging, trauma, alkaline burns, infection and previous surgeries lead to stress in corneal endothelium and affect density and morphology. Researchers (8, 13) reported that decompensation would develop when endothelial cell count falls below 300/mm² and decompensation risk would be high if cell count is between 300-500. In the present study, while endothelial cell count is 2797 cells/mm^2 in group I it was measured as 2001 cells/mm^2 in group II and 2625 cells/mm^2 in group III. This condition indicates that although alkaline burns lead to decrease in endothelial cell count of cornea, autologous serum administration provides increments in alkaline burns-induced reductions in this cell count. However cell count did not fall under 300 cells which are the critical level stated by researchers, in both groups.

In conclusion, in this study, autologous serum administration was detected to reduce the endothelial defective areas and be effective in normalizing morphologic structures of cells through fluorometric and fluorescence microscopic examinations in experimentally-induced alkaline burn model.

References

1. Campos CF, Jorge AT, Talieri IC, et al. Ocular alkali lesions in dogs. Acetylcysteine and blood serum effects. Braz J Vet Res Anim Sci 2003; 40: 36-44.
2. Petrotsos GJ, Courtyl R, Petroutsos G. Comparison of the effects of egf, pfgf and edgf on corneal epithelial wound healing. Curr Eye Res 1984; 3: 593-598.
3. Salman IA, Gundogdu C. Epithelial healing in experimental corneal alkali wounds with nondiluted autologous serum eye drops. Cutan Ocul Toxicol 2010; 29: 116-121.
4. Singh G, Foster CS. Epidermal growth factor in alkali-burned corneal epithelial wound healing. Am J Ophthalmol 1987; 103: 802-807.
5. Bahn CF, Glassman RM, Mc Callium DK, et al. Postnatal development of corneal endothelium. Invest Ophthalmol 1986; 27: 44-51.
6. Bourne WM, Hodge DO, Nelson LR. Corneal endothelium five years after transplantation. Am J Ophthalmol 1994; 118: 185-196.
7. Chung JG, Fagerholm P. Endothelial healing in rabbit corneal alkali wounds. Acta Ophthalmol 1987; 6: 648-656.
8. Tadeu JA, Abib FC, Pereira GT, et al. Density of corneal endothelial cells in eyes of dogs using specular of microscopy. Bras J Vet Res Anim Science 2006; 43: 476-480.
9. Gunay C, Sagliyan A, Yaman M. Intraocular silicon prosthesis in dogs. Indian Vet J 2005; 82: 844-846.
10. Gurelik G, Akata F, Bilgihan K, Hasanreisoglu B. Silicon-Induced corneal endothelial changes. J Retina-Vitreous 1986; 6: 50-55.
11. Tuft SJ, Coster DJ. The corneal endothelium. Eye 1990; 4: 389-424.
12. Laing RA, Neubauer L, Kayne HL, Leibowitz HM. Evidence for mitosis in the adult corneal endothelium. Ophthalmol 1984; 91: 1129-1134.
13. Matsuda MM, Sawa HF, Edelhauser SP, Bartels AH, Kenyon KR. Celular migration and morphology in corneal endothelial wound repair. Investig Ophthalmol Vis Sci 1985; 26: 443-449.
14. Traffers WF. Human corneal endothelial wound repair; in vitro and in vivo. Ophthalmol 1982; 89: 605-613.
15. Akyol SI. Effects of autologous serum eye drops on corneal wound healing after superficial keratectomy in rabbits. Cornea 2006; 25: 1178-1181.
16. Fox RI, Chan R, Michelson JB, Belmont JB, Michelson PE. Benedfical effect of artifical tears made with autologous serum in patients with keratokonjunctivitis sicca. Arthritis Rheum 1984; 27: 549-561.
17. Mahajan PS. Autologous serum in eye disorders. JK Science 2009; 11: 111-113.
18. Ohashi Y, Motokura M, Kinoshita Y. Presence of epidermal growth factor in human tears. Invest Ophthalmol 1989; 30: 1879-1887.
19. Tsuboto K, Goto E, Schimmura S, Shimazaki J. Treatment of persistent epithelial defect by autologous serum application. Ophthalmol 1999; 106: 1984-1989.
20. Van-Setten G, Tervo T, Tarkhanen A. Epidermal growth factor (egf) in ocular fluids: persence, orgin and therapeutic considerations. Acta Ophthalmol Supp 1992; 202: 54-59.
21. Iguchi I, Komiyama K. Enhancing effect of platalet-derived growth factor on migration of corneal endothelial cells. Cornea 1995; 14: 365-371.
22. Gordon SR. The localization of actin in dividing corneal endothelial cells demonstrated with nitrobenzoxadiazole phallacidin. Cell Tissue Res 1983; 229: 533-539.
23. Kim EK, Kim HB, Chung YT, Kim IC. Endothelial f-actin changes in the alkali burned rabbit cornea. Yonsei Med J 1994; 34: 484-492.
24. Fujino Y, Tanishima T. Actin in wound-healing of rabbit corneal endothelium. II. Study by nitrobenzoxadiazole-phallcidin method. Jpn J Ophthalmol 1987; 31: 393-404.