RESEARCH ARTICLE

F.U. Vet. J. Health Sci.
2020; 34 (3): 165 - 171
http://www.fusabil.org

Investigation of the Antioxidant Effects of Amniotic Fluid on Corneal Alkali Burns *

Cihan GÜNAY [1, a]
Aydın SAĞLIYAN [1, b]
Seval YILMAZ [2, c]
Eren POLAT [1, d]
Emre KAYA [2, e]
Mehmet Cengiz HAN [1, f]
Kemal Kenan KARABULUT[1, g]

[1] University of Fırat,
Faculty of Veterinary Medicine,
Department of Surgery,
Elazığ, TURKEY

[2] University of Fırat,
Faculty of Veterinary Medicine,
Department of Biochemistry,
Elazığ, TURKEY

[a] ORCİD: 0000-0002-0476-6164
[b] ORCİD: 0000-0002-8226-0740
[c] ORCİD: 0000- 0001-5472-3560
[d] ORCİD: 0000- 0002-3999-1310
[e] ORCİD: 0000- 0002-7445-3091
[f] ORCİD: 0000- 0001-9178-6261
[g] ORCİD: 0000- 0002-2934-964X

In the study, 40 female Wistar Albino rats were used to investigate the healing effects of amniotic fluid on corneal alkali burns. Rats were randomly divided into 4 groups of an equal numbers of rats. The groups were assigned as follows: The control group; the group “AF”, in which the rats were applied with bovine amniotic fluid for 20 days; the group “AB+SF”, in which an alkali burn was formed in the right eyes of the rats and saline was applied for 20 days; and the group “AB+AF”, in which an alkali burn was formed in the right eyes of the rats and amniotic fluid was applied for 20 days. At the end of the 20th day of the experiment, malondialdehyde (MDA) and glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activity were determined in the corneal tissue of the animals euthanized. The MDA levels and the GSH-Px activity in the corneal tissue were found to be increased significantly in the “AB+SF” group compared to the control group. When the MDA levels in the corneal tissue were compared between the “AB+AF” and the “AB+SF” groups, a statistically significant decrease was determined and the MDA levels approached to those obtained in the control group. The GSH levels in the corneal tissue were lower in the “AB+SF” group compared to the control group; the difference between these two groups was found to be significant. When the GSH levels in the corneal tissue were compared between the “AB+AF” and the “AB+SF” groups, a statistically significant increase was determined and the values approached the control group. In conclusion, oxidative stress may play a role in corneal alkali burns, and amniotic fluid may be effective in improving it.

Key Words: *Amnion, burn, cornea, rat*

Alkali Kornea Yanıklarında Amniyotik Sıvının Antioksidan Etkisinin Araştırılması

Çalışmada, amniyotik sıvının korneal alkali yanıkları üzerindeki iyileştirici etkilerini araştırmak için 40 dişi Wistar Albino sıçan kullanıldı.Sıçanlar rastgele ve eşit sayıda sıçandan oluşan 4 gruba ayrıldı. Gruplar aşağıdaki gibi tasarlanmıştır: Kontrol grubu; sıçanlara 20 gün boyunca sığır amniyotik sıvısının uygulandığı “AF” grubu; sıçanların sağ gözünde bir alkali yanığın oluştuğu ve 20 gün boyunca FTS uygulandığı “AB+SF” grubu; ve sıçanların sağ gözünde bir alkali yanığın oluştuğu ve 20 gün boyunca amniyotik sıvı uygulandığı “AB+AF” grubu.Deneyin 20. gününün sonunda ötenazi edilen hayvanların kornea dokusundaki malondialdehit (MDA) ve glutatyon (GSH) düzeyleri ile glutatyon peroksidaz (GSH-Px) aktivitesi tespit edildi. Kornea dokusundaki MDA seviyeleri ve GSH-Px aktivitesi, “AB + SF” grubunda kontrol grubuna göre anlamlı olarak arttı. Kornea dokusundaki MDA düzeyleri ‘‘AB + AF’’ ve ‘‘AB + SF’’ grupları arasında karşılaştırıldığında istatistiksel olarak anlamlı bir düşüş tespit edildi ve MDA düzeyleri kontrol grubunda elde edilenlere yaklaştı. Kornea dokusundaki GSH düzeyleri, ‘‘AB + SF’’ grubunda kontrol grubuna göre daha düşüktü ve bu iki grup arasındaki farkın anlamlı olduğu görüldü. Kornea dokusundaki GSH düzeyleri ‘‘AB + AF’’ ve ‘‘AB + SF’’ grupları arasında karşılaştırıldığında istatistiksel olarak anlamlı bir artış tespit edildi ve değerler kontrol grubuna yaklaştı. Sonuç olarak, oksidatif stres korneal alkali yanıklarda rol oynayabilir ve amniyon sıvısı onu iyileştirmede etkili olabilir.

Anahtar Kelimeler: *Amniyon, yanık, kornea, rat*

Introduction

Inflammation of the cornea, which is characterized by tissue loss both in the corneal epithelium and stroma is called ulcus cornea (corneal ulcer) and it does not heal spontaneously. Causes of a corneal ulcer (ulcus cornea) are divided into two groups, as exogenous and endogenous. Often, eye trauma is considered to be a major cause of ulcus cornea formation in domestic animals. Sometimes, endogenous causes are also observed (1, 2).

Bacteria rarely cause corneal ulcers although they have a primary role in corneal infections. Entropion, ectropion, distichiasis, keratoconjunctivitis sicca, foreign body sting in the cornea, conjunctival lithiasis, eyelid tumors, chemical burns, ectopic ciliate, canine juvenile disease, mycotic factors, and immune system disorders can also cause ulcus cornea (3, 4).

The corneal epithelium is highly resistant to infections when the eye is not traumatized and the integrity of the cornea is not compromised. When there is an injury due to direct or indirect factors disrupting the integrity of the epithelium, the passage of microorganisms through the corneal epithelium is facilitated and this may lead to

Received : 04.02.2020
Accepted : 18.09.2020

Yazışma Adresi
Correspondence

Eren POLAT
University of Fırat,
Faculty of Veterinary Medicine,
Department of Surgery,
Elazığ – TURKEY

erenpolat@firat.edu.tr

* This study (VF.16.11) was supported by Firat University Scientific Research Projects.

corneal ulceration. If corneal defects are not treated early or if there is no appropriate treatment; ulcus cornea may develop in a very short period of time, reaching the deep layers of the cornea and leading to major complications such as corneal vascularization, uveitis, panophthalmia or even corneal perforation (2, 5, 6).

Treatment can be medical or surgical. In order to reduce the pain and treat the infection; locally applied medications, anticollagenase drugs (acetylcysteine, cysteine, sodium EDTA, calcium EDTA, progesterone, medroxyprogesterone, sodium ascorbate, sodium citrate, penicillamine, tetracycline, thiol peptides, aprotinin and polysulfate glycosaminoglycan, heparin, and autogenous blood serum), and vitamins (A, B_2, B_{12}, C) are administered to patients as effective modes of medical treatment (2, 4, 7-9).

Amniotic membrane and amniotic fluid accelerate epithelization, prevent protein and fluid loss at the wound surface, increase fibroblastic activity, and reduce adhesion formation; as well as having antibacterial, antioxidant, and non-immunological effects (10-13).

The amniotic membrane was first used by Davis in 1910 for skin transplantation. The use of amniotic membranes in ophthalmology was first introduced by De Rotthile. The researcher used a newly acquired chorion layer in 1940 for the treatment of conjunctival epithelial defects, but no significant success was achieved. Later studies have shown that amniotic fluid and amnion membranes have been successfully used in conjunctiva pterygium surgery, filtering surgery, and symblepharon therapy, and for the treatment of chemical burns, periocular surface neoplasms, bleb leaks, conjunctival chalazion, entropion, and cicatrizing conjunctivitis (12, 13).

Free oxygen radicals (FOR) have been reported to play a major role in the development of tissue damage and disease in many organs, including the eye. Despite the diversity, widespread distribution, and highly effective activities of antioxidant molecules against the free radicals in cells and tissues; this defense system may sometimes become insufficient; resulting in macromolecular damage. The commonly known FOR include hydrogen peroxide (H_2O_2), superoxide (O_2), and hydroxyl radicals (OH^-) These molecules cause cellular damage by reacting with lipids, proteins, and DNA (14-19).

The aim of this study was to determine the presence of FOR and antioxidant capacity in the corneal alkali burns and to evaluate the effects of amniotic fluid on healing.

Materials and Methods

Design of the Experimental Groups: The study was initiated after receiving the official approval of the Firat University Animal Experiments Ethics Committee (20.04.2016, Karar no: 82). Animal material in the study consisted of 40 female, adult, Wistar Albino rats weighing 250-300 grams, which were obtained from Fırat University Experimental Research Center. The rats were kept in special cages and under special laboratory conditions (24±3℃, 40-60% humidity, and 12 hours of darkness and 12 hours light in a day). Standard pellet feed was used for feeding. Rats were randomly divided into 4 equal groups. Group I rats were selected as the control group and no interventions were performed. Rats in the second (II) group were administered bovine amniotic fluid (AF) for 20 days (2 drops 3 times a day). No other procedures were applied to this group. The third (III) group was selected as the positive control group and a lesion of alkali burn was created in the right eyes of the rats. Saline was applied to these rats for 20 days (AB+SF) (2 drops 3 times a day). Alkali burn was formed in the right eye of the rats in the fourth (IV) group and bovine amniotic fluid was applied for 20 days (AB+AF) (2 drops 3 times a day).

In the rats with an alkaline burn; anesthesia was achieved with intramuscular injections of 4 mg/kg xylazine hydrochloride (Rompun; 23.32 mg/mL, Bayer, Istanbul, Turkey) and 50 mg/kg ketamine hydrochloride (Ketalar, 50 mg/mL, Eczacibasi, Istanbul, Turkey). Alkali burns were induced only in the right eye of the anesthetized rats. For this purpose, a 3 mm-diameter filter paper impregnated with 2 N NaOH was left on the cornea for 40 seconds. After exposure to the alkaline agent, the eyes were washed with saline for 2 minutes.

Preparation of Amniotic Fluid: The amniotic fluid used in the experiment was collected after cesarean operations of healthy pregnant cows. The collected amniotic fluid was centrifuged at 2000 rpm for 15 minutes, and the supernatant, which was formed above the tube at the end of the centrifugation, was placed in a sterile tube and stored at +4 ºC.

Euthanasia: To obtain biochemical data, the animals were euthanized with carbon dioxide inhalation in a closed box at the end of the 20th day. Malondialdehyde (MDA), reduced glutathione (GSH) levels and glutathione peroxidase (GSH-Px) activities at cornea tissue were determined after removing the cornea from euthanized animals. Prior to the analyses, the collected cornea tissues were washed with physiological saline solution, diluted at a ratio of 1:10 with distilled water, and homogenised in a Potter-Elvehjem homogeniser (CAT R50D, Germany). The homogenate was centrifuged at +4 °C (at 3.000 g for 15 min to quantify MDA and GSH; and at 10.000 g for 55 min to test the amount of GSH-Px).

Determination of MDA Levels in the Corneal Tissue: The MDA assay was performed according to the method modified by Placer et al. (20). This method is based on the reaction of thiobarbituric acid (TBA) with MDA, which is one of the aldehyde products of lipid peroxidation (LPO). The resulting MDA forms a pink complex with TBA, and the absorbance of this solution was assessed by spectrophotometry at 532 nm to determine the LPO level.

Determination of GSH Levels in the Corneal Tissue: The GSH assay was performed by the method of Ellman et al (21), in which the quantification of 5.5'-dithiobis- (2-nitrobenzoic acid) (DTNB) is performed via

the enzymatic cycle procedure in the presence of nicotinamide adenine dinucleotide phosphate (NADPH) and glutathione reductase (GR). This method is a spectrophotometric method based on the highly stable yellow color of sulfhydryl groups when DTNB is added.

Assay of GSH-Px Activity in the Corneal Tissue: Beutler (22) method was used for measuring the corneal tissue GSH-Px activity. GSH-Px catalyzes the oxidation of GSH to the oxidized glutathione (GSSG) using H_2O_2. The rate of GSSG synthesis is measured by evaluating the GR reaction. One mole of GSSG is produced for the reduction of each molecule of t-butyl hydroperoxide (t-BOOH) in the reaction medium. The reduction of GSSG to GSH is catalyzed by the GR enzyme. In this reaction, 1 mole of NADPH is oxidized for the reduction of each mole of GSSG. The GSH-Px activity is calculated by spectrophotometry by the reduction of the optical density (OD) of the system at 340 nm after NADPH oxidation.

Protein Assay in Biological Fluids: The amount of protein in the homogenate samples was measured according to the modified Lowry (23) method; in which the alkaline copper tartrate separator formed complexes with peptide bonds. Each group of 7 or 8 amino acids binds 1 atom of Copper (Cu). Phenol separator gives a purple-blue color when added into the Cu-treated mixture. This color intensity was read at 650 nm wavelength. Since there was not a linear relationship at high concentrations between the concentration of protein and the color, samples were diluted and measured.

Statistical Analysis: The statistical significance among the groups was analyzed using the SPSS 22 software package. The Shapiro-Wilk normality test was applied to determine whether the raw values of all of the measured parameters showed a normal distribution. It was found that the values of all parameters conformed to a normal distribution. Based on the results of the Shapiro-Wilk normality test, one-way analysis of variance (ANOVA) was used for determining the differences among the groups. The post hoc Tukey test was used for among comparisons. The data obtained as a result of the study were expressed as mean and standard error. Values of $P<0.05$ were accepted as statistically significant (24).

Results

Clinical Observation: On the tenth day-examination; corneal ulceration, diffuse conjunctivitis, and blepharospasm were detected in 6 rats, corneal perforation was detected in one rat in Group III (AB+SF was applied). Deep corneal opacity, diffuse conjunctivitis, and photophobia were detected in one rat. Reduction in corneal opacity was present in one rat in addition to the observed improvement in its general condition. Indirect ophthalmoscopy showed deep vascularity in the cornea (Figure 1).

On the tenth-day control examinations in Group IV, in which AB+AF was applied; photophobia, corneal opacity, ulceration, and conjunctivitis were seen in 3 rats. Reductions in corneal opacity, photophobia, and very mild conjunctivitis; as well as symptoms of recovery, were detected in seven rats in Group IV (Figure 2). On the tenth day examinations, no negative findings were observed in the first and second groups (Figure 3 and 4).

On the twentieth-day examinations in Group III (AB+SF); advanced corneal ulceration, corneal edema, photophobia, and advanced level of conjunctivitis were observed in 6 rats in addition to corneal perforation detected in two rats in the previous examination. In this group, one rat showed diffuse corneal opacity, photophobia, dry eye, and conjunctivitis. In one rat, there was a moderate improvement since this rat had moderate corneal opacity. Indirect ophthalmoscopy showed deep vascularity in the cornea (Figure 5).

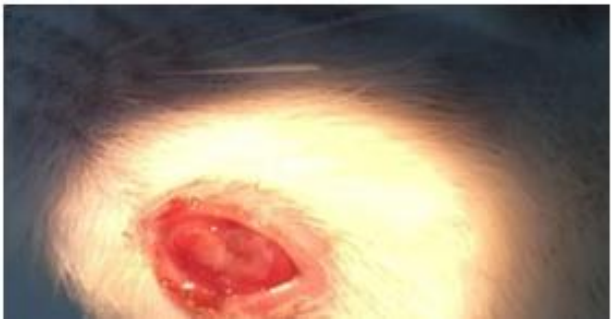

Figure 1. AB+SF group (10. day)

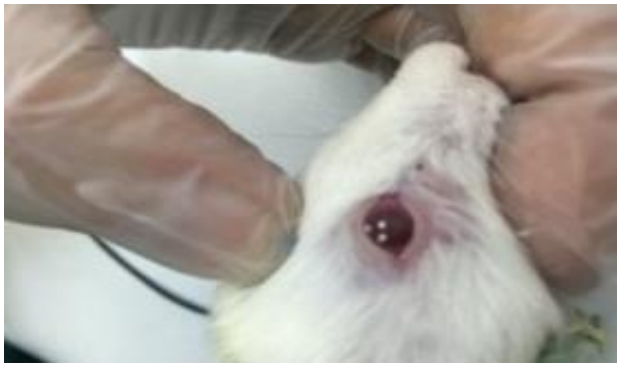

Figure 2. AB+AF group (10. day)

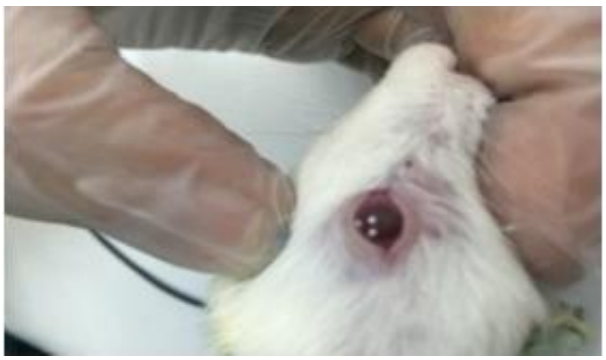

Figure 3. Control group (10. day)

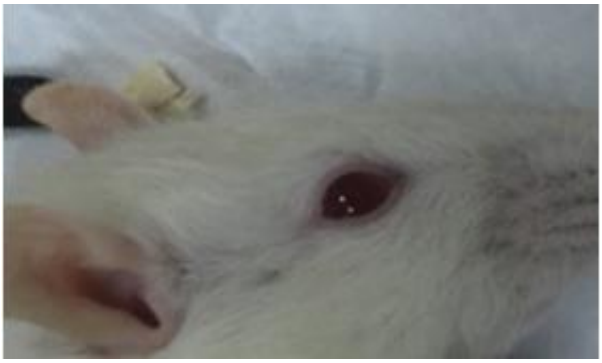

Figure 4. Control group 2 (10. day)

On the twentieth-day control examinations in Group IV (AB+AF); corneal ulceration, photophobia, corneal edema, and conjunctivitis persisted in one rat while another rat had moderate corneal opacity, photophobia, and moderate conjunctivitis. Clinically satisfactory symptom improvements were observed in 8 rats. Blurring in the cornea has completely disappeared but photophobia and conjunctivitis persisted (Figure 6).

On the twentieth-day control examinations in the first and second groups, no negative symptoms were observed (Figure 7-8).

Results of the Biochemical Analysis: The MDA level in the corneal tissue was higher in the "AB+SF" group compared to the control group and the difference between these two groups was statistically significant ($P<0.001$). When only AF applied group was compared with the control group; no statistically significant differences were detected. When the corneal MDA levels of the "AB+AF" group were compared with those of the "AB+SF" group; a statistically significant decrease was detected and the final values were found similar to the values of the control group ($P<0.001$) (Table 1).

The GSH level in the corneal tissue was lower in the AB+SF applied group compared to the control group and the difference between these two groups was statistically significant ($P<0.001$). When the AF applied group and AB+AF applied groups were compared with the control group; no statistically significant differences were found. When the corneal GSH levels of the corneal tissue in the AB+AF applied group were compared with those of the "AB+SF" group; a statistically significant increase was detected and final values were found to be similar to the values measured in the control group ($P<0.001$) (Table 1).

The GSH-Px activity in the corneal tissue was higher in the (AB+SF) applied group compared to the control group and the difference between these two groups was found to be statistically significant ($P<0.001$). When the AS group and the "AB+AF" applied groups were compared with the control group; no statistically significant differences were found. When the corneal GSH-Px activity of the AB+AF applied group was compared to that of the AB+SF group; a statistically significant decrease was detected and the final values were found to be similar to the values of the control group ($P<0.001$) (Table1).

Figure 5. AB+SF group (20. day)

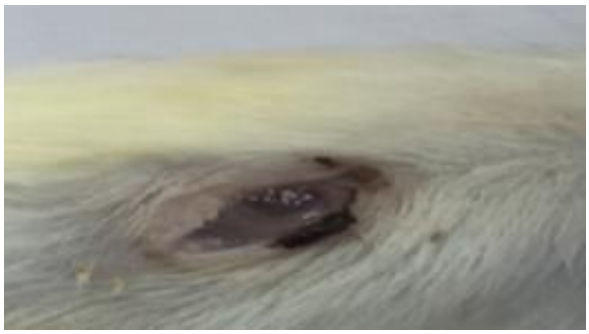

Figure 6. AB+AF group (20. day)

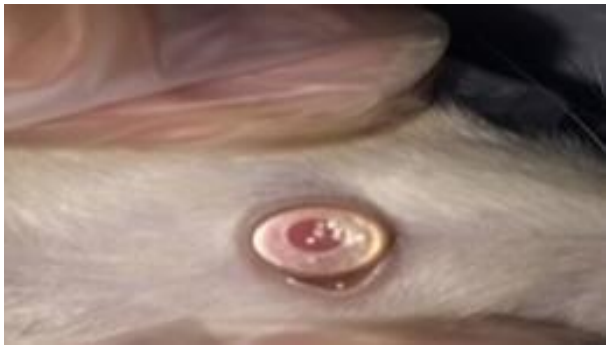

Figure 7. Control group (20. day)

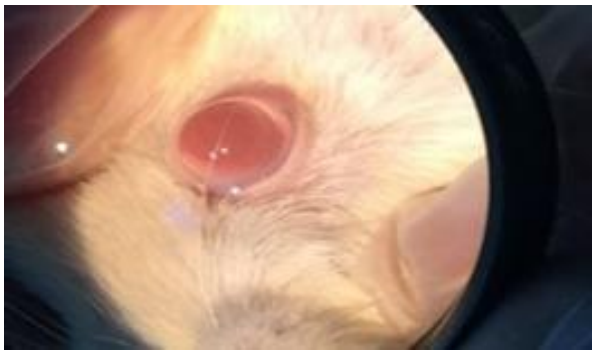

Figure 8. Control group 2 (20. day)

Table 1. Statistical analysis of biochemical measurement values in corneal tissue

	Control	Amnotic Fluid (AF)	Alkali Burn+Saline (AB+SF)	Alkali Burn+Amniotic Fluid (AB+AF)	P
MDA (nmol/g tissue)	0.51±0.02[a]	0.52±0.01[a]	0.71±0.01[b]	0.54±0.01[a]	0.001
GSH (µmol/mL)	4.62±0.12[a]	4.34±0.12[a]	3.24±0.14[b]	4.39±0.24[a]	0.001
GSH-Px (U/mg protein)	0.30±0.01[a]	0.28±0.01[a]	0.54±0.03[b]	0.29±0.02[a]	0.001

[a.b] Different letters on the same line show differences between groups (P<0.001)

Discussion

The damage and inflammation occurring after alkali burns in the cornea may have a possibility of development of permanent defects after some time. Amniotic membrane transplantation and amniotic fluid reduce the inflammation and the effects of the injury when applied early in the course of the tissue damage and these interventions accelerate epithelization (2, 5, 7, 8, 13). Amniotic membrane and fluid show these effects via several growth factors and they display antioxidant effects (10, 13).

Cornea has enzymatic and nonenzymatic antioxidant and repair systems that counteract the effects of free radicals to maintain cell integrity. From the perspective of the balance between the oxidant and antioxidant systems, it is apparent that the integrity of the cell membrane is impaired in the traumatized cells, resulting in the emergence of the oxidative stress conditions in the injured tissue due to the release of oxidants such as O_2 or H_2O_2 (25-34). Oxidative stress is characterized by elevated FOR, leading to lipid peroxidation in membranes. Lipid peroxidation affects important cellular structures and functions by causing molecular damages in the organism. Alkali burns promote the synthesis of FORs in cornea and the FORs cause cellular damage. After severe alkali burns in the cornea, it was reported that MDA levels, the most important product of lipid peroxidation in aqueous humor, was increased and activity of superoxide dismutase (SOD), GSH-Px, which is the enzymatic scavenger of free radical, was decreased (33). Thus, increased MDA levels in the tissue indicate an increase in FOR. In this study, biochemistry tests showed that oxidative stress was found out to be increased in the groups with alkali burns. FORs can be released from the burnt cells of the necrotic tissue with consequent decreases in antioxidants in the area of tissue damage after the alkaline burn. It has been documented that acute oxidative stress, inflammation, and corneal neovascularization play an important role in corneal damage and even in visual loss due to a chemical burn (35).

Antioxidants provide basic support for ameliorating the effects of injury in cells. Some researchers (28, 32, 33) have studied new pharmacological antioxidants to counteract the adverse effects of oxidant substances for improving the disease prognosis. Other researchers (27, 29, 33) investigate the efficiency of endogenous antioxidants in the saliva and autogenous serum. Öner et al., (25) found that antioxidant levels decreased significantly in localized burns and local antioxidant treatments eliminated tissue damage in these injuries. In this study, it was determined that exogenous bovine amnion fluid was effective on corneal alkali burns; the MDA levels decreased; and the GSH levels and GSH-Px activities increased significantly in the group receiving amnion fluid therapy with alkaline burn. Eye diseases, including corneal burns, are accompanied by overproduction of FORs and depletion of endogenous antioxidants. It is known that antioxidants such as GSH, SOD, catalase, and GSH-Px are highly effective free radical scavengers of FORs. These enzymes have been shown to be effective in the treatment of various ocular diseases associated with oxidative stress (28, 30, 31, 34). In an experimental study, Nirankari et al., (35) created alkali burns in the anterior segment of rabbit eyes. The study demonstrated that alkali burns impaired the normal response to free radicals and that the resulting impairment could be controlled by early treatment with exogenous SOD. In the anterior segment of the rabbit eye, MDA levels were increased in the 4th, 24th, and 72nd hours after the alkali burn and the SOD activity was significantly reduced. It was reported that after subconjunctival injection of exogenous SOD, the MDA levels were decreased and the endogenous SOD activity was significantly increased. The study demonstrated that bovine amnion fluid improved corneal alkali burns and had antioxidant properties as it increased the levels of MDA.

It has been suggested that GSH, one of the intracellular antioxidants, is susceptible to tissue damage, and has a regulator role especially in the early stages of wound healing. In the study of Otto et al. (34) the intracellular amount of GSH was increased by treating the wounded epithelial cells with GSH and the authors concluded that; compared to the control group, the wound healing was higher in the group, to which this intervention was applied.

GSH-Px is an important corneal defense enzyme that protects against H_2O_2 and lipid peroxides (36). Steiling et al. (26) showed an increase in GSH-Px activity in combination with oxidative stress in scar tissue during wound healing in their study. After alkaline burns, lipid peroxidation and GSH-Px activity in the cornea increased significantly compared to normal control animals in that study. For this reason, the cornea becomes more susceptible to the harmful effects of FOR after alkali burns. In the “AB+AF” applied group, the level of the GSH-Px activity almost returned to the levels of the control group due to epithelial healing in the cornea. The increase in the GSH-Px activity that catalyzes the

conversion of GSH to GSSG (oxidized glutathione) may have accelerated the conversion of GSH to GSSG. GSH is a major cellular -SH (sulfhydryl group) compound that interacts with numerous electrophilic and oxidizing compounds. It is a nucleophile and an effective reducing agent. It can serve as a non-enzymatic antioxidant via the direct interaction between the -SH group and FOR or it may be involved in the enzymatic detoxification reaction of FOR as a coenzyme.

Investigators (1, 4, 7) stated that surgical techniques such as corneal transplantation or conjunctivoplasty can be performed to treat corneal alkali burns or corneal ulcers caused by exogenous causes if medical treatment can not be provided for 3 weeks. Duschesne (27) reported that corneal integrity was achieved in more than two weeks by using amniotic membranes with human fibrin glue in a patient where corneal damage was large or even the cornea was perforated. Sridhar et al. (37) stated that after 2 weeks of treatment for chemical burns in the acute phase, the ocular damage had disappeared but that a four-month period had to pass in order to ensure complete recovery. In this study, corneal epithelial defects were resolved after 20 days of treatment, as supported by biochemical findings, too. In this regard, it has been seen that researchers have provided a satisfactory improvement in the health care process.

Some researchers (38, 39) used bovine amniotic fluid and bovine amniotic membrane in acute alkaline corneal burns experimentally created. In these studies, as in our study, reductions in areas where corneal opacity is concentrated and reductions in epithelial defects were found.

In conclusion; there are many treatment options for corneal alkali burns and corneal opacity, ulcer, vascularization, and inflammatory findings related to alkali burn. In this study; it was supported by the clinical and biochemical findings that amniotic fluid could resolve complications in the cornea compared to the control group. Besides, it is considered to be an advantage that the amniotic fluid is easy to obtain, use, and store.

References

1. Saroglu M, Arıkan M. Researches on the comparision of various anticollagenase drugs in the treatment of experimentally induced corneal alkali burns in rabbits. J Fac Vet Med Istanbul Univ 2002; 28: 287-300.
2. Kenyon K. Inflammatory mechanisms in corneal ulceration. Trans Am Ophthalmol Soc 1985; 83: 610-663.
3. Shahriari HA, Tokhmechi F, Reza M, Hashermi NF. Comparison of the effect of amniotic membrane suspension and autologous serum on alkaline corneal epithelial wound healing in the rabbit model. Cornea 2008; 27: 1148-1150.
4. Christmas, R. Management of chemical burns of the canine cornea. Can Vet J 1991; 32: 608-612.
5. Arora R, Mehta D, Jain V. Amniotic membrane transplantation in acute chemical burns. Eye 2005; 19: 273-278.
6. Kozak I, Trbolova A, Sevcikova Z, Juhas T, Ledecky V. Superficial keratectomy, limbal autotransplantation and amniotic membrane transplantation in the treatment of severe chemical burns of the eye. Acta Vet Brno 2002; 71: 85-91.
7. Gunay C, Sagliyan A, Yilmaz S, et al. Evaluation of autologous serum eyedrops for the treatment of experimentally induced corneal alcali burns. Revue Méd Vét 2015; 166: 63-71.
8. Gunay C, Sagliyan A, Ozkaraca M, Han MC. Effect of autologous serum on healing of corneal endothelium in experimentally-induced alkaline burns of the cornea in rabbits. FÜ Sağ Bil Vet Derg 2013; 27: 31-34.
9. Choi JA, Choi JS, Joo CK. Effects of amniotic membrane suspension in the rat alkali burn model. Mol Vis 2011; 17: 404-412.
10. Sato H, Shimazaki J, Shinozaki N. Role of growth factors for ocular surface reconstruction after amniotic membrane transplantation. Inv Ophtalmol Vis Sci 1998; 39: 428.
11. Saw VPJ, Minassian D, Dart JKG. Amniotic membrane transplantation for ocular disease: A review of the first 233 cases from UK user group. Br J Ophthalmol 2007; 91: 1042-1047.
12. Stridar MS, Bansal AK, Sanqvan VS, Rao GN. Amniotic membrane transplantation in akute chemical and thermal injury. Am J Ophthalmol 2000; 130: 134-137.
13. Kim JS, Kim JC, Na BK, Jeong JM, Song CY. Amniotic membrane patching promotes healing and inhibits proteinase activity on wound healing following acute corneal alkali burn. Exp Eye Res 2000; 70: 329-337
14. Fantone JC, Ward PA. Role of oxygen-derived free radicals and metabolites in leukocyte-dependent inflammatory reactions. Am J Pathol 1982; 107: 395.
15. Freeman BA, Crapo JD. Biology of disease: Free radicals and tissue injury. Lab Invest 1982; 47: 412-426.
16. Rangan U, Bulkley GB. Prospects for treatment of free radical-mediated tissue injury. Br Med Bull 1993; 49: 700-718.
17. Kehrer JP. Free radicals as mediators of tissue injury and disease. Crit Rev Toxicol 1993; 23: 21-48.
18. Carubelli R, Nordquist RE, Rowsey JJ. Role of active oxygen species in corneal ulceration. Effect of hydrogen peroxide generated in situ. Cornea 1990; 9: 161-169.
19. Yilmaz S, Kaya E, Comakli S. Vitamin E (α tocopherol) attenuates toxicity and oxidative stress induced by aflatoxin in rats. Adv Clin Exp Med 2017; 26: 907-917.
20. Placer ZA, Cushman L, Johnson BC. Estimation of products of lipid peroxidation in biological fluids. Anal Biochem1966; 16: 359-364.
21. Ellman GL, Courtney KD, Andres V, Featherstone RM. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem Pharmacol 1961; 7: 88-95.
22. Beutler E. Red cell metabolism. A manual of biochemical methods. 2nd Edition, New York, NY, USA: Grune and Starton; 1984.

23. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the folin-phenol reagent. J Biol Chem 1951; 193: 265-257.

24. Karagöz Y. SPSS 22 Uygulamalı Biyoistatistik. Güncellenmiş 2. Basım, Ankara: Nobel 2015.

25. Oner M, Dulgeroglu TC, Karaman I, et al. The effects of human amniotic fluid and different bone grafts on vertebral fusion in an experimental rat model. Curr Ther Res Clin Exp 2015; 77: 35-39.

26. Steiling H, Munz B, Werner S, Brauchle M. Different types of ROS scavenging enzymes are expressed during cutaneous wound repair. Exp Cell Res 1999; 247: 484-494.

27. Duschesne B, Tahi H, Galand A. Use of human fibrin glue and amniotic membrane transplant in corneal perforation. Cornea 2001; 20: 230-232.

28. Aslan R, Dündar Y. Serbest radikal giderici maddelerin yara iyileşmesi üzerine etkileri. İnsizyon 2000; 3: 74-79.

29. Gakhramanov FS. Effect of natural antioxidants on antioxidant activity and lipid peroxidation in eye tissue of rabbits with chemical burns. Bull Exp Biol Med 2005; 140: 289-291.

30. Salman IA, Kızıltunc A, Baykal O. The effect of alkali burn on corneal glutathione peroxidase activites in rabbits. Turkish J Med Sci 2011; 41: 483-486.

31. Sun Y, Oberley LW, Li YA. Simple method for clinical assay of superoxide dismutase. Clin Chem 1988; 34: 497-500.

32. Williams DL. Oxidative stress and the eye. Vet Clin North Am Small Anim Pract 2007; 38: 179-192.

33. Yuan HP, Lu SR, Wang BI. An experimental study of treatment with superoxide dismutase for alkali burn in the anterior segment of the rabbit eye. Chinese J Opthal 1994; 30: 50-52.

34. Otto WR, Rao J, Cox HM, et al. Effects of pancreatic spasmolytic Polypeptide (PSP) on epithelial cell function. Eur J Biochem 1996; 235: 64.

35. Nirankari VS, Varma SD, Lakhanpal V, Richards RD. Superoxide radical scavenging agents in treatment of alkali burns: An experimental study. Arch Ophthalmol 1981; 99, 886-887.

36. Atalla LR, Sevenian A, Rao NA. Immunohistochemical localization of glutathione peroxidase in ocular tissue. Curr Eye Res 1988; 7: 1023-1027.

37. Srithar MS, Bansal AK, Sanqvan VS, Rao GN. Amniotic membrane transplantation in akute chemical and thermal injury. Am J Ophthalmolgy 2000; 130: 134-137.

38. Gonenci R, Altug ME, Koc A, Oksuz H, Yuksel H. Effects of the bovine amniotic membrane on corneal healing acute alkali burns in rabbits. J Anim Vet Adv 2009; 8: 1653-1659.

39. Gonenci R, Altug ME, Koc A, Yalcin A. Effects of bovine amniotic fluid on acute corneal alkali burns in rat. J Anim Vet Adv 2009; 8: 617-623.