Autologus serum and sodium hyaluronate role in alkali corneal burn healing

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Background/aim
Corneal burn wound healing includes a sequence of difficult processes that are focused on improving the outcomes, in particular, the healing time and the feature of the scar. Bodily fluids such as natural tears and autologus serum were used in the treatment of corneal burn. The aim of the study was to investigate the cure of corneal burn alkali injury by autologous serum (AS) and/or sodium hyaluronate.
Materials and methods
A total of 50 Wister rats (200–250 g) were distributed into five groups: group I acted as the control, group II had alkali burn (AB) with sodium hydroxide, group III had AB treated with AS, group IV was AB treated with sodium hyaluronate, and group V had AB with sodium hydroxide treated with autologus serum and sodium hyaluronate. Comet assay analysis was applied for cornea to determine DNA damage. Malondialdehyde, superoxide dismutase, glutathione peroxidase, and catalase were measured to estimate the balance between oxidants and antioxidants in the cornea.
Results
The data indicated a significant increase ($P<0.05$) in all comet assay parameters and malondialdehyde level in addition to a significant decrease ($P<0.05$) in superoxide dismutase, glutathione peroxidase, and catalase activity owing to AB. Enhancements of measured parameters were observed in all other treated groups.
Conclusion
AS accelerates the AB healing process, but the process was faster when diluted by sodium hyaluronate.

Keywords:
alkali burn, autologus, cornea, malondialdehyde, rats

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Introduction

The cornea is a transparent layer that allows light to pass through and reach the inside of the eye. The transparent corneal epithelium avoids any damage to the lower layers of the cornea by acting as a barrier against damage. Any injury to this layer may affect its transparency and decrease its protective capacity [1]. Corneal ulcers are a common ophthalmologic disease. It is caused by using of contact lenses, trauma, infection, and chemical injuries [2]. Chemical injury such as alkali burn (AB) is one of the sources of oxidative stress in the eye that produces free radicals. Lipid peroxidation results from oxidative stress owing to interaction of oxygen-derived free radicals with polyunsaturated fatty acids. Malondialdehyde (MDA) and 4-hydroxynonenal are derived from the products of lipid peroxidation [3].

Natural tears consist of complex mixtures of water, hydrocarbons, proteins, salts, and lipids that artificial tears cannot precisely substitute [4,5]. Moreover, many used artificial tear solutions have chemical additives (preservatives) to avoid contamination, which stimulates toxicity and allergic reactions [6].

The use of autologous serum (AS) to treat ocular AB began in 1970 [7]. To date, it has been used for the treatment of different diseases of the ocular surface, such as persistent epithelial defects [8], neurotropic keratopathy [9], superior limbic keratoconjunctivitis [10], and chemical injuries. Human serum has strong likeness to natural tears and contains many important components, including (a) epithelial growth factor, which accelerates epithelial cell migration and has antiapoptotic effects; (b) transforming growth factor-β (TGFβ), involved in the epithelial and stromal repair process; (c) vitamin A, which may prevent epithelial squamous metaplasia and modulate the expression of thrombospondin 1; (d) thrombospondin 2, vascular endothelial growth factor A, and metallopeptidase 9, which together with TGFβ promote wound healing; (e) albumin, which has antiapoptotic activity; (f) α-2 macroglobulin, which exhibits anticollagenase activity; and (g) fibronectin,

which is important in cell migration [11]. Akcam *et al.* [12] clarified that using the AS can reduce epithelial healing duration after the application of photo-refractive keratectomy. Jeng and Dupps [13] established that a concentration of 100% AS has a very high percentage of serum proteins, but the rate of venipuncture and the amount of blood needed are twice with the use of 100% AS drops, so 50% AS drops in the eye were better and safer.

Sodium hyaluronate is the salt of hyaluronic acid and is found in a variety of connective tissues of humans. Hyaluronic acid has immunosuppressant, anti-inflammatory, and antiapoptotic effects at the tissue level [14]. Sodium hyaluronate is used as a late-release system for autologus serum [15]. The AS diluted with sodium hyaluronate was better tolerated by patients and chosen owing to its effect on tear stability [16]. The aim of the present study was to explore the treatment of corneal burn alkali injury by AS and/or sodium hyaluronate.

Materials and methods

Chemicals

Chemicals utilized were obtained from Sigma Company (St. Louis, Missouri, USA) with the highest purity commercially available.

Animals

Wister rats of both sexes (200–250 g) were randomly chosen from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The protocol of the experiment was approved by the local ethical committee, which was applied by the Association for Research in Vision and Ophthalmology. Fifty rats were divided into five groups:

(1) Group I acted as the control.
(2) Group II had AB and termed as AB.
(3) Group III had AB and was treated with AS eye drops for four times a day immediately after AB until decapitation and was termed as AB+AS.
(4) Group IV had alkaline burn and was treated with 0.1% sodium hyaluronate eye drops for four times daily after AB until decapitation and was termed as AB+SH.
(5) Group V had alkaline burn and was treated with AS and sodium hyaluronate eye drops (mix in a ratio of 1 : 1) as the same protocol of groups III and IV and termed as AB+AS+SH.

All groups were decapitated after 21 days and then eyes were extracted and corneas were obtained from rats via cutting through the ora serata. Five corneas from all animal's groups were weighed and then used for comet assay. The rest of the five corneas were homogenized using cell homogenizer (Homogenizer type 7400 Tübingen, Edmund Biihler, W. Germany), in a 10-fold volume of 20 mmol/l ice cold tris-HCl buffer, pH: 7.4. The homogenate was centrifuged for 20 min at 10 000 rpm in a bench centrifuge (Awel centrifuge MS 20; Centrifuge type Awel, Chateau Gontier, France). The resultant supernatant was used for detection of oxidants–antioxidants parameter measurements.

Corneal alkali burn

The rats were anesthetized via the intraperitoneal injection of a mixture of ketamine 80 mg/kg and xylazine 7 mg/kg. For the AB model, a piece of filter paper measuring 0.3 mm in diameter was soaked in 0.01 mol/l NaOH and applied to the center of the cornea of the right eye for 45 s followed by rinsing with 0.1 ml sterile saline [17].

Autologous serum preparation

It was prepared from rats by withdrawing blood from the tail veins. A volume of 1.5 ml of blood was centrifuged for 5 min at 1500 rpm as described by Tsubota *et al.* [18]. The serum was separated in a sterile bottle coated with a substance that cuts out ultraviolet light to prevent degradation of vitamin A and kept in a refrigerator at 4°C for use.

Comet analysis

The previously weighted corneas were minced with a tweezer in Hanks' balanced salt solution-buffer, and the comet assay was performed with the resulting cell suspension on the same day. Alkaline comet assay, also called alkaline single cell gel electrophoresis assay, was conducted as previously described by Mohanty *et al.* [19]. Komet 5 image analysis software developed by Kinetic Imaging Ltd. (Liverpool, UK) linked to a charge-coupled device camera was used to assess the quantitative and qualitative extents of DNA damage in the corneal cells by measuring the length of DNA migration, the percentage of migrated DNA, and tail moment by observing 50–100 randomly selected cells per sample. The tail length was measured from the middle of the nucleus to the end of the tail. The percentage of DNA in the tail was calculated from the fraction of DNA in the tail divided by the amount of DNA in the nucleus multiplied by 100. The tail moment is defined as the product of the tail length and the fraction of total DNA in the tail [20].

Biochemical analyses

The protein value of the clear supernatants from rat's corneas of all groups was studied by using the Lowry

method [21] to use in calculation of antioxidants levels. MDA levels (nmol/mg), superoxide dismutase (SOD; Ul/mg), glutathione peroxidase (GSH-Px; mIU/mg), and catalase (CAT; IU/mg) enzyme activities were measured in the supernatants.

MDA levels were measured using the MDA Assay Kit (MAK085) according to the thiobarbituric acid reactive substances method [22]. SOD activity was measured as described before by Durak *et al.* [23] using SOD assay kit (K335-100) (Cell Biolabs, Inc., USA). One unit of SOD activity was expressed as the enzyme protein amount causing 50% inhibition in the nitro blue tetrazolium reduction rate. CAT activity was determined using Bio Vision's Catalase Assay Kit (K773-100) by measuring the absorbance decrease of H_2O_2 at 240 nm [24]. GSH-Px activity was measured by Cellular Activity Assay Kit following the changes in the nicotinamide adenine dinucleotide phosphate absorbance at 340 nm [25]. In the activity calculations, extinction coefficients of H_2O_2 and nicotinamide adenine dinucleotide phosphate were used for CAT and GSH-Px enzymes, respectively.

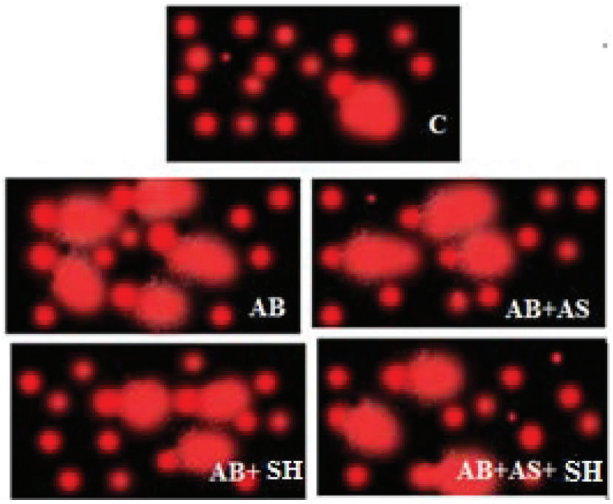
Statistical analysis

Data were presented as the mean±SD. The analysis of variance and the paired *t*-test were employed using a commercially available software package (SPSS 11 for windows; SPSS Inc., Chicago, Illinois, USA). All results were calculated to be significant at *P* value less than 0.05.

Results

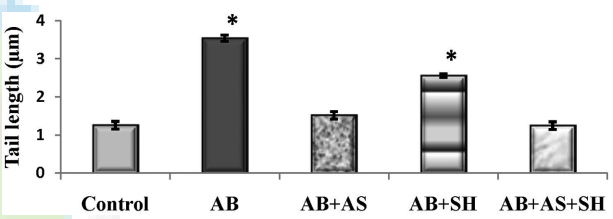
The present results obtained in Fig. 1 show the image of comet assay in corneas of rat groups: control group of rats (C), AB group, AB treated with AS group (AB+AS), AB treated with sodium hyaluronate eye drops (AB+SH), and AB treated with AS and sodium hyaluronate eye drops (AB+AS+SH). Normal DNA cell was characterize by a circular shape, but the damaged cell appeared with a tail and differed in length and percentage of DNA concentrated in it according to the degree of damage. The qualitative examination of the comet assay image revealed four parameters illustrated in Figs 2–5. Tail length, % tail DNA, tail moment, and % tailed cells were calculated for the different groups and found to be 1.260±0.1, 1.362±0.1, 1.716±0.1, and 6±1 µm, respectively, for the control group, and 3.541±0.08, 4.025±0.04, 14.253±0.05, and 17±1.5 µm, respectively, for AB corneas group, which exhibited significant increase ($P<0.05$) than the control group. After AS treatment (AB+AS group), the value of comet parameters restored to normal value, indicating insignificant difference than control group. Moreover, the same results appeared for AB+AS +SH rat group treated with autologous and sodium hyaluronate eye drops. AB+SH group indicated a significant increase in all comet parameters compared with control, but at the same time, their values were better than AB group.

Figure 1



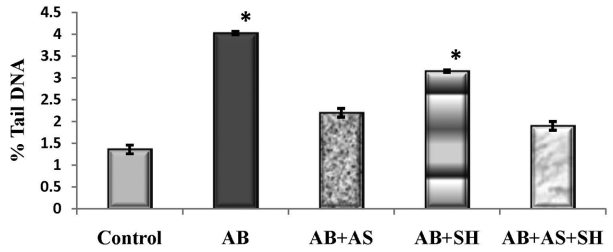
Photograph of comet assay electrophoresis of cornea in all rats groups. C is the control group, AB is alkali burn group, AB+AS is alkali burn treated with autologus serum, AB+SH is alkali burn treated with sodium hyaluronate eye drops, and AB+AS+SH is alkali burn treated with autologus serum and sodium hyaluronate eye drops. AB, alkali burn; AS, autologous serum; SH, sodium hyaluronate.

Figure 2



Tail length in µm for AB and treated groups with AS and/or SH eye drops compared with control. AB, alkali burn; AS, autologous serum; SH, sodium hyaluronate. *$P<0.05$.

Figure 3



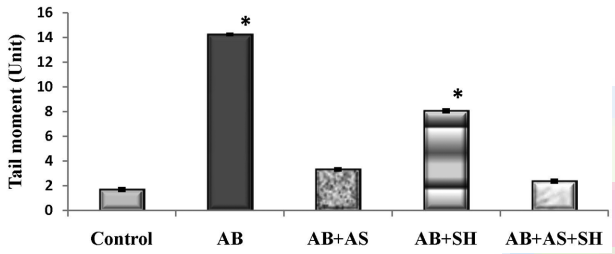
Percentage tail DNA for AB and treated groups with AS and/or SH eye drops compared with control. AB, alkali burn; AS, autologous serum; SH, sodium hyaluronate. *$P<0.05$.

Oxidants and antioxidants parameters

MDA level is usually used as an indicator of free radical-induced lipid peroxidation injury. MDA level in corneal tissue were measured and illustrated in Table 1. The results indicated a significant increase ($P<0.05$) in MDA level in rats' corneas after AB by sodium hydroxide and then returned to the control value after treatment with AS. Sodium hyaluronate eye drops treatment led to significant increase ($P<0.05$) in MDA value compared with control and also a significant decrease ($P<0.05$) compared with AB without treatment. Reduction of MDA level was observed for AB+AS+SH group, in a manner that mimicked the control value.

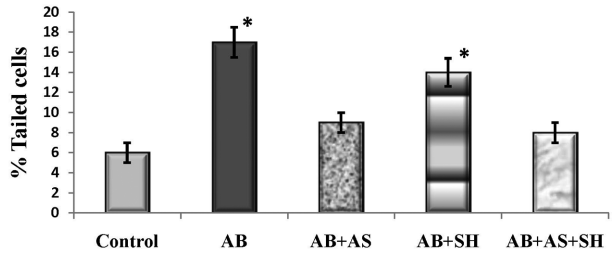
SOD, GSH-Px, and CAT activity revealed a significant decrease ($P<0.05$) after AB (AB group) compared with the control group. Action of AS treatment appeared in group AB+AS by the absence of any significant change in SOD, GSH-Px, and CAT activity owing to autologus serum treatment. A significant decrease ($P<0.05$) was observed for AB+SH group in these parameters compared with control group, and in the same context, a significant increase ($P<0.05$) compared with the AB group. Activity of SOD, GSH-Px, and CAT returned to their normal value in AB+AS+SH owing to treatment with both autologus and sodium hyaluronate eye drops.

Figure 4



Tail moment (unit) for AB and treated groups with AS and/or SH eye drops compared with control. AB, alkali burn; AS, autologous serum; SH, sodium hyaluronate. *$P<0.05$.

Figure 5



Percentage of tailed cells for control and alkali burn with or without different treatment. *$P<0.05$.

Discussion

Chemical injuries to the eye account between 11.5 and 22.1% of total ocular injuries [26]. Among the most serious ocular injuries is ABs of the cornea. Normal corneas have well-developed antioxidant defense systems – such as glutathione peroxidase, SOD, and CAT – which contain direct free radical scavenging activity after corneal injuries. An imbalance between the antioxidants and prooxidants in the cornea results in oxidative stress as well as activation of the lipid peroxidation, increased MDA levels, and decreased antioxidants enzymes activities.

In our study, the ROSs produced by AB affect the balance between the oxidation and antioxidant systems, finally leading to DNA damage. The alkaline comet assay is a sensitive technique for direct imaging of DNA single-strand breaks on the level of a single cell [27]. We found that AB could be a reason for DNA single-strand breaks as shown by tail moment of alkaline comet assay. In addition, it was found that AB had an effect on DNA division as indicated by the tail moment and tail length of alkaline comet assay (in comparison with the control group).

We conducted the study to assess the efficacy and safety of treatment of corneal burn alkali injury by AS and/or sodium hyaluronate.

Early studies have established that sodium hyaluronate, used in ophthalmology in the form of artificial tears for treating dry eye disease, shows rheological

Table 1 Malondialdehyde value and superoxide dismutase, glutathione peroxidase, and catalase activities in cornea for all studied groups

	MDA (nmol/mg)	SOD (U/mg)	GSH-pX (mIU/mg)	CAT (IU/mg)
Control	0.26±0.05	21.94±2	39.22±1	4.52±0.02
Alkali burn	0.45±0.04[a]	14.05±1[a]	28.25±2[a]	2.82±0.03[a]
Alkali burn+autologous serum	0.30±0.03	18.95±1	35.90±2	3.97±0.08
Alkali burn+sodium hyaluronate	0.38±0.04[a]	16.36±1[a]	32.86±1[a]	2.99±0.04[a]
	0.32±0.03	19.82±1	36.45±2	4.14±0.02

All data are presented as mean±SE. CAT, catalase; GSH-pX, glutathione peroxidase; MDA, malondialdehyde; SOD, superoxide dismutase. [a]Significant difference than control group at $P<0.05$.

characteristics and an adherence to epithelium ability that increase the bioavailability of beneficial agents on the ocular surface more than other viscosity agents such as hypromellose [28].

An unpreserved artificial tear containing 0.1% sodium hyaluronate was initiated to be useful in the enhancement dry eye symptoms with a significant improvement in the mean tear film osmolarity and corneal staining scores. However, the use of artificial tears has some limitations [28]. Artificial tears lack the accountability of the complex composition of the natural tear film. Natural tears have a compound composition of water, salts, hydrocarbons, proteins, and lipids that artificial tears cannot accurately replicate [29].

AS eye drops suggest a possible benefit over usual therapies on the hypothesis that AS eye drops only serve as a lacrimal substitute to offer lubrication but contain other biochemical constituents that allow them to copy natural tears. This could be partially confirmed by some studies [30,31] that AS for the recovery of corneal tissue resulted in dramatic improvement in the ocular surface, but artificial tears could not.

The activities of antioxidant enzyme and MDA data revealed improvement in AB+AS group and AB+AS +SH group than AB+SH group, and the results are supported by Gunay *et al.* [32] who found that the activities of all of these enzymes showed enhancement in AS and AS in combination with SH than sodium hyaluronate eye drops alone, which suggests the protective action of AS as an antioxidant in opposition to the oxidative stress induced by the alkali chemical injury. Another study by Gus *et al.* [33] concluded that 50% AS eye drops seem to contain total reactive antioxidant potential concentrations that are approximately five to six times greater than in the natural tears of young and healthy individuals.

The major weakness of AS treatment is the necessity for blood donation, so discovering the most favorable dilution of serum, in isolation or mixture with conventional therapeutic measures, may well reduce the amount of blood needed [34]. Undiluted serum eye drops are also stated to supply elevated concentration of growth factors as well as inhibit probable toxicity of diluents and any infectivity through the dilution process [35].

However, the major disadvantages of using undiluted serum eye drops are the problem of repeated blood draws, large volume of blood collection, and potential ocular exasperation accompanying with the extra viscosity of the eye drops. The deposit of immunoglobulin in the cornea and the existence of corneal peripheral infiltrates with 100% AS may permanently the chance that serum may include components caused detrimental to the ocular surface [36]. TGFβ, for example, has antiproliferative effects, and elevated concentrations of TGFβ may inhibit wound healing of the ocular surface epithelium [37]. This was one of the reasons for using a diluted solution of serum to keep the TGFβ levels equivalent to tears. Therefore, even if 100% AS drops were more active, some studies supposed 50% AS eye drops were safer and more manageable.

This development was more obvious and considerable in the group that used AS in combination with sodium hyaluronate. Corneal tissue from AB group treated with a combination of 0.1% SH and AS showed significantly less tail moment and tail length than those treated with AS only, so most preferred to continue treatment with this AS combined with sodium hyaluronate. SH was an efficient protective agent that had antioxidant properties and could decrease DNA damage and cell apoptosis induced by AB as previously mention by López-García *et al.* [16].

Conclusion

AS accelerates the AB healing process by increasing the activities of the antioxidants enzymes and decreasing MDA, which improve the oxidative stress induced by AB. Moreover, AS combined with sodium hyaluronate seems to be a promising agent for clinical use in cornea alkali wound healing owing to its effects in decreasing DNA damage and had more efficient protective antioxidants properties.

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Nil.

Conflicts of interest

There are no conflicts of interest.

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