Antioxidant Activity of Tear Fluid in Experimental Alkali Eye Burns

O. V. Gulidova, O. B. Lyubitskii*, G. I. Klebanov*, and N. B. Chesnokova

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Antioxidant activity of the tear fluid in intact rabbits and after alkali burn of the cornea was evaluated by measuring chemiluminescence in a hemoglobin—hydrogen peroxide—luminol system. After alkali burn of the cornea antioxidant activity of tear fluid decreased starting from day 3 and reached its minimum on days 7-21. This decrease correlated with the depth of ulceration of the corneal stroma. This method allow to specify indications for antioxidant therapy in corneal disease and to choose the most effective antioxidants.

Key words: tear fluid; antioxidant activity; chemiluminescence; alkali burn of the cornea

Studies of tear fluid is a highly informative noninvasive method used in ophthalmology. Tears contain biologically active components and play an important role in the protection of anterior eye structures. Tear antioxidants are an essential part of this defensive system which protects the surface eye structures against free radical oxidation (FRO) under normal and pathological conditions. Published data suggest that, tear fluid contains a variety of antioxidants: ascorbic acid (3.9-23 mg/dl) [9], non-protein SH-groups, γ-glutamyltranspeptidase [6], glutathione, glutathione reductase, Cu, Zn superoxide dismutase (SOD) (103±32 ng/ml soluble protein) [8], Ca²⁺ and lactoferrin [3], as well as urate, albumin, and ceruloplasmin.

Damage and inflammation in the cornea are accompanied by massive release of oxidants and free oxygen radicals into the tear fluid from corneal and conjunctival cells and activated neutrophils and macrophages migrating into the tear fluid during inflammation. Antioxidant activity (AOA) of tears is characterized by inhibition of FRO of various substrates. Natural and synthetic antioxidants are widely used for the treatment of various corneal diseases. However, little is known about antioxidation potency of tears under different pathological conditions. For instance, chemiluminescence (ChL) methods revealed reduced AOA of the tear fluid in patients with pathological myopia, diabetes, and open angle glaucoma.

ChL is a direct method for studying free radicals and relevant reactions [2]. It is widely used for evaluation of the functional state and production of active oxygen species by blood and tissue phagocytes responsible for their protective function. In the present study we investigated AOA of the tear fluid by the kinetics of luminol-activated chemoluminescence in a hemoglobin—hydrogen peroxide—luminol system (Hb—H₂O₂—Lm) [4,5]. In our experimental model, chemoluminescence is emitted during oxidation of luminol by free radicals generated in the presence of hemoglobin and hydrogen peroxide. The resultant AOA in the system of Hb—H₂O₂—Lm depends on contributions of different hydrophilic antioxidant components of the tear and their interactions.

Our aim was to study AOA of rabbit tear fluid in a Hb—H₂O₂—Lm system under normal conditions and in the dynamics of corneal burn diseases caused by alkali.
MATERIALS AND METHODS

Experiments were performed on 10 Chinchilla rabbits (20 eyes) weighing 2.5-3.0 kg. Alkali burn of the cornea (stage III burn of standard area and depth) was produced by applying 7-mm cotton disks soaked with 10% NaOH on the central area of the cornea. After 40 sec the disks were removed, and the eye was rinsed with 20 ml physiological saline. Alkali burns were inflicted against the background of subcutaneous anesthesia with 2.5% chlorpromazine and 0.5% diazepam (1:1) in a volume of 0.5 ml/kg body weight and local instillation of 0.5% tetracaine.

Tear fluid was collected from intact animals and on days 1, 3, 7, 14, 21, and 28 after corneal injury. The fluid was collected with filter paper disks placed for 5 min into the conjunctival sac. Tear components were eluted with physiological saline and centrifuged to remove insoluble components, the supernatant was stored before tests.

Examination of rabbit eyes was performed on days 3, 7, 14, 21, and 28 after injury using a binocular microscope. Conjunctival (eyelid edema, hyperemia, and discharge) and corneal symptoms (injection and neovascularization assessed by the length and density of newly formed vessels penetrating the cornea, as well as infiltration, total area and depth of erosions) were scored. The presence of exudate in the anterior eye chamber was determined. The state of the cornea was assessed using 0.5% fluorescein which positively stains deepithelialized regions.

Tear AOA was determined by measuring ChL kinetics in the Hb—H₂O₂—Lm model system [5]. Reaction medium (500 μl) contained 0.3 μM Hb and 10 μM luminol in phosphate buffer (50 mM KH₂PO₄, 100 μM EDTA, pH 7.4). Luminol-dependent FRÖ was stimulated with hydrogen peroxide (30 μM). ChL was measured on a Biotoks-7 chemiluminometer (Energia, Moscow) connected to a personal computer. The latency of ChL, i.e. the period from initiation of FRO to appearance of ChL, was determined. AOA of tear eluate was calculated as the ratio of ChL latencies determined in the presence of tear (T) and in the model system (T₀): \( AOA = \frac{T}{T₀} \).

Hemoglobin (Sigma) and luminol (ICN) were used.

RESULTS

First, the effect of tear eluate on the kinetics of ChL in the Hb—H₂O₂—Lm system was studied. Typical ChL kinetic curves in the absence and presence of tear eluate (200 μl) are presented in Fig. 1. Addition of tear eluate into the system prolonged the latency of ChL compared with the control and this increase directly correlated with the volume of added eluate (Fig. 1, insert). This effect of tears was similar to that of the serum and main serum antioxidants on this model system [4]. Therefore, the latency of ChL may be used as a characteristic of AOA in the tear fluid.

The cornea represents a vessel-free structure, which consists primarily of the connective tissue (stroma) with layers of collagen fibers. The distance between these fibers determines transparency of the cornea, while distortions can cause its opacity. The stroma is coated with Bowman’s membrane with stratified epithelium (anterior) and Descemet’s membrane (posterior) with single-layer epithelium (corneal endothelium). Alkali burn caused immediate deep epithelialization of the injured area and corneal edema which decreases only on days 10-14 after injury. Within 2-3 days after injury the deep epithelialized surface was usually covered with new epithelium and blood vessels grow into the cornea from the limb. Rejection of newly formed epithelium and ulceration of the corneal stroma led to deep ulcers, which were formed on days 14-21 after burn and sometimes reached the internal basement membrane culminating in descemetocele and perforation of the cornea. Twenty-one days after burn, a scar formed on the injured surface and the vessels were emptied. In most cases, the injured cornea was totally covered with epithelium by day 28. The state of the cornea (erosion area and depth of ulceration) was scored according to a 4-point scale.

We found that despite standard procedure of burn modeling, the dynamics of healing was different in different animals and even in different eyes of the rel. units

![Figure 1](image-url)