Use of Natural Antioxidants for the Correction of Changes in General and Local Parameters of Lipid Peroxidation and Antioxidant Defense System during Experimental Eye Burn

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The effect of natural antioxidants in grade III chemical eye burn was studied in experiments on rabbits at various stages of burn disease. The use of histochrome, Gingko Biloba, and diquertin in combination with complex drug therapy decelerated the decrease in the antioxidant potential of tear fluid and blood plasma. This treatment was also followed by a decrease in the concentration of end products of free radical oxidation.

Key Words: *lipid peroxidation; antioxidants; eye burn disease; antioxidant potential of tear fluid and blood plasma*

Burns are the most severe damage to organs of vision [2].

Here we studied the role of lipid peroxidation (LPO) and antioxidant defense system during experimental chemical burn of grade III. The effectiveness of natural antioxidants in correcting the consequences of this disorder was evaluated.

MATERIALS AND METHODS

Experiments were performed on 29 rabbits (58 eyes). The animals were divided into 7 groups: groups 1-6, treatment (4 specimens per group); and group 7, control (n=5).

The solution of histochrome was instilled to group 1 rabbits 2 h after burn. Histochrome was administered into the eye (0.02% histochrome, 2 drops in each eye, 6 times a day), subconjunctivally (0.02% histochrome, 0.3-0.4 ml daily for 7 days), and in the ear vein (1.0 ml 1.0% histochrome in 10.0 ml physiological saline, daily drop infusions). This treatment was performed in combination with standard antiinflammatory therapy, which included instillation of 30% sodium sulfacyl (2 drops, 6 times a day) and 0.25% levomycetin (2 drops, 6 times a day), application of tetracycline ointment into the conjunctival fornix (3 times a day), and subconjunctival injection of gentamicin (0.4 ml daily for 14 days).

Group 2 rabbits received standard antiinflammatory drugs and antioxidant flavonoid Gingko Biloba in a daily dose of 120 mg for 4 weeks. Group 3 rabbits were treated with antioxidant flavonoid diquertin in a daily dose of 120 mg for 4 weeks. Gingko Biloba in a dose of 120 mg/kg was given perorally to group 4 rabbits for 1 month. Group 5 rabbits received histochrome (instillations and subconjunctival injections) and diquertin. Group 6 rabbits received combination therapy with histochrome (0.02 and 1.0%), Gingko Biloba, and diquertin. Physiological saline (2 drops, 6 times a day; 0.4 ml subconjunctivally) was instilled to rabbits of control group 7 for 1 month.

Tear fluid was collected using filter paper circles placed in the lower conjunctival sac for 5 min. Tear components were eluted with physiological

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saline. The eluate was centrifuged. The supernatant was used for further studies.

The blood (2 ml) was collected from the ear vein, placed in tubes with 125 U/ml heparin, and centrifuged at 900*g* for 10 min.

Antioxidant activity (AOA) of tear fluid and plasma was measured using a model system of hemoglobin, hydrogen peroxide, and luminol [3,4]. AOA was estimated by recording the kinetics of chemiluminescence in a model system of hemoglobin, hydrogen peroxide, and luminol. The reaction medium (500 μg) contained 0.3 μM hemoglobin and 10 μM luminol in phosphate buffered saline (50 mg KH_2PO_4 and 160 μM EDTA, pH 7.4). H_2O_2 (30 μM) was added to initiate free radical oxidation of luminol. The measurements were performed on a Biotoks-7 chemiluminometer (Energiya).

RESULTS

In group 1 rabbits inflammation of the anterior segment of the eye disappeared after 9.8±3.4 days. The severity of corneal edema in these animals decreased on day 10.8±2.4. The cornea was completely epithelialized after 16.8±3.2 days. Corneal transparency returned to normal in 2 eyes. Grade I and II leukoma was found in 5 and 1 eyes, respectively.

In group 2 rabbits inflammation of the anterior segment of the eye disappeared after 14.5±1.8 days. The severity of corneal edema in these animals decreased on day 13.5±2.7. The cornea was completely epithelialized after 19.2±2.4 days. Corneal transparency returned to normal in 1 eye. Grade I and II leukoma was found in 4 and 3 eyes, respectively.

In group 3 rabbits inflammation of the anterior segment of the eye disappeared after 18.2±2.9 days. The severity of corneal edema in these animals decreased on day 17.2±4.1. The cornea was completely epithelialized after 22.6±3.4 days. The animals had grade I (4 eyes) and grade II (4 eyes) leukoma.

In group 4 rabbits inflammation of the anterior segment of the eye disappeared after 8.2±3.2 days. The severity of corneal edema in these animals decreased on day 9.3±2.7. The cornea was completely epithelialized after 14.2±2.2 days. Corneal transparency returned to normal in 4 eyes. Grade I and II leukoma was found in 3 and 1 eyes, respectively.

In group 5 rabbits inflammation of the anterior segment of the eye disappeared after 8.6±3.7 days. The severity of corneal edema in these animals decreased on day 9.4±2.2. The cornea was completely epithelialized after 16.2±3.4 days. Corneal transparency returned to normal in 3 eyes. Grade I and II leukoma was found in 3 and 2 eyes, respectively.

In group 5 rabbits inflammation of the anterior segment of the eye disappeared after 7.8±2.7 days. The severity of corneal edema in these animals decreased on day 6.2±2.3. The cornea was completely epithelialized after 12.8±4.8 days. Corneal transparency returned to normal in 4 eyes. Grade I leukoma was found in 4 eyes.

In group 7 rabbits (control) inflammation of the anterior segment of the eye disappeared after 28.3±5.7 days. The cornea was completely epithelialized after 30.8±5.6 days. Corneal perforation was observed in 4 eyes. Corneal ulceration and descemetocele were revealed in 2 eyes. Grade IV leukoma was found in 4 eyes.

The antioxidant potential of tear fluid was measured before the incidence of burn. AOA of tear fluid and blood plasma was 9.30±0.57 and 42.9± 8.8 μM, respectively. The concentration of TBA-reactive substances (malonic dialdehyde, MDA) in blood plasma was 0.24±0.18 nmol/mg lipids.

In group 1 rabbits the antioxidant potential decreased insignificantly. AOA of tear fluid decreased to 7.25±2.20, 7.6±4.8, and 8.82±5.60 μM on days 3 (necrotic stage of burn disease), 14 (stage of dystrophy), and 30 (stage of leukoma formation and cicatrization), respectively. AOA of blood plasma from these rabbits was 40.8±7.6, 39.9±6.8, and 41.2±5.8 μM on days 3, 14, and 30, respectively. Plasma MDA concentration was 0.25±0.12, 0.26± 0.03, and 0.25±0.04 nmol/mg lipids on days 3 (necrotic stage of burn disease), 14, and 30, respectively. These data indicate that the use of natural antioxidant histochrome in combination with standard antiinflammatory drugs led to inhibition of free radical oxidation and LPO. The antioxidant potential of tear fluid and blood plasma decreased insignificantly in group 1 animals.

AOA of tear fluid from group 2 rabbits was 7.90±0.38, 7.70±0.05 ($p<0.05$ compared to the control), and 9.10±0.08 μM ($p<0.05$ compared to the control) on days 3, 14, and 30 after the incidence of burn, respectively. During the necrotic stage of burn disease (days 3) AOA of blood plasma from these animals was 38.90±1.29 μM (*vs.* 42.9±8.8 μM in the control, $p<0.05$). AOA of blood plasma from group 2 rabbits was 35.9±8.9 ($p<0.05$) and 42.8± 5.8 μM on days 14 and 30 after the incidence of burn, respectively. MDA concentration was 0.26± 0.03 ($p<0.05$), 0.30±0.04, and 0.26±0.12 nmol/mg lipids on days 3, 14, and 30 after chemical burn, respectively (*vs.* 0.24±0.18 nmol/mg lipids in the control).

The total antioxidant potential of tear fluid from group 2 rabbits receiving Gingko Biloba in combination with standard antiinflammatory drugs de-

creased by 15.20±1.08% compared to control animals. AOA of tear fluid from these rabbits decreased by 27.4±3.28 and 14.40±2.12% on days 14 (stage of dystrophy) and 30, respectively.

We compared the therapeutic effectiveness of histochrome and Gingko Biloba. Histochrome was more potent that Gingko Biloba in stabilizing AOA of tear fluid and blood plasma during the necrotic stage (by 3 and 1.3 times, respectively). Histochrome decreased the concentration of MDA in blood plasma by 8 times. At the stage of dystrophy, histochrome decreased AOA of tear fluid (by 1.2 times) and blood plasma (by 1.8 times) and concentration of MDA (by 5 times). At the stage of leukoma formation and cicatrization, plasma AOA and MDA concentration in histochrome-receiving rabbits were lower than in animals of the Gingko Biloba group (by 1.1 and 1.5 times, respectively).

AOA of the tear fluid from group 3 rabbits was 6.50±0.43, 5.20±0.37, and 8.50±2.34 μM on days 3, 14, and 30 after the incidence of burn, respectively. AOA of blood plasma from these animals was 35.6±4.2 ($p<0.05$), 33.8±2.8 ($p<0.05$), and 42.90±3.45 μM on days 3, 14, and 30 after the incidence of burn, respectively. Plasma MDA concentration was 0.27±0.03 ($p<0.05$), 0.32±0.01 ($p<0.05$), and 0.26±0.08 nmol/mg lipids on days 3, 14, and 30, respectively.

The total antioxidant potential of blood plasma from group 3 rabbits decreased by 22.40±1.38%.

Hence, peroral administration of diquertin to rabbits over 1 month after the incidence of burn stabilized AOA of tear fluid and blood plasma. This treatment was followed by a decrease in the concentration of MDA in blood plasma. At the necrotic stage of burn disease, diquertin was less potent than Gingko Biloba in modulating AOA of tear fluid and plasma (by 2 and 1.8 times, respectively). However, diquertin was more potent than Gingko Biloba in stabilizing the concentration of MDA in blood plasma (by 2.3 times). During the stage of dystrophy (day 14), AOA of tear fluid and blood plasma in diquertin-receiving rabbits decreased more significantly than in animals of the Gingko Biloba group (by 1.6 times). However, the concentration of TBA-reactive substances in blood plasma from diquertin-receiving rabbits was 1.3-fold higher compared to animals of the Gingko Biloba group. These differences were found during all stages of burn disease.

Total AOA of tear fluid from group 5 rabbits decreased to 8.00±0.22, 8.00±0.38, and 9.00±0.47 μM on days 3, 14, and 30, respectively.

The total antioxidant potential of blood plasma from group 5 rabbits was 40.90±0.68, 39.0±4.5, and 42.6±4.8 μM on days 3, 14, and 30, respectively.

MDA concentration increased to 0.25±0.11, 0.26±0.09, and 25.50±0.05 nmol/mg lipids on days 3, 14, and 30, respectively.

Combined treatment with histochrome and diquertin was followed by a decrease in total AOA of tear fluid by 14.1±3.2, 24.80±3.25, and 21.40±1.32% on days 3 (necrotic stage of burn disease), 14 (stage of dystrophy), and 30 (stage of leukoma formation and cicatrization), respectively.

AOA of blood plasma from group 5 rabbits decreased by 3.80±1.24, 11.80±2.32, and 4.20±0.08% on days 3, 14, and 30 after the incidence of burn, respectively. The concentration of TBA-reactive substances in blood plasma increased by 5.00±0.26, 16.7±1.32, and 5.00±1.01% on days 3, 14, and 30, respectively.

Our results show that combined administration of histochrome and diquertin neutralized products of free radical oxidation and LPO. This treatment stabilized the antioxidant potential of tear fluid and blood plasma. A correlation was found between biochemical parameters and clinical signs of eye burn.

The total antioxidant potential of tear fluid from group 6 rabbits slightly decreased on day 3 after the incidence of burn (8.90±0.68 μM). A less significant decrease in AOA of tear fluid was found on day 14 (8.70±0.78 μM). The total antioxidant potential increased to 9.00±0.58 μM on day 30. The total antioxidant potential of blood plasma from these animals was 41.3±4.8, 40.20±3.47, and 42.6±5.2 μM on days 3, 14, and 30 after the incidence of burn, respectively. MDA concentration increased to 0.25±0.07 and 0.26±0.03 nmol/mg lipids on days 3 and 14 after the incidence of burn, respectively. MDA concentration decreased to 0.25±0.03 nmol/mg lipids on day 30.

Combined administration of histochrome, Gingko Biloba, and diquertin contributed to the decrease in total AOA of tear fluid to 3.80±0.18, 6.60±1.32 (stage of dystrophy), and 1.00±0.02% (stage of leukoma formation and cicatrization).

The total antioxidant potential of blood plasma from group 6 rabbits decreased by 3.80±0.18, 6.60±1.32, and 2.20±0.02% on days 3, 14, and 30, respectively. Plasma MDA concentration in these animals increased by 5.00±1.12 and 8.00±1.28% on days 3 and 14, respectively. Plasma MDA concentration was 0.24±0.18 nmol/mg lipids on day 30.

We conclude that chemical eye burn is accompanied by a decrease in the antioxidant potential of tear fluid and blood plasma. The concentration of TBA-reactive substances in blood plasma increases

under these conditions. The severity of these changes is maximum at the stage of trophic disturbances. The antioxidant potential of tear fluid and blood plasma decreases less significantly after treatment with antioxidants histochrome, Gingko Biloba, and diquertin in combination with drug therapy at all stages of eye burn. Administration of natural antioxidant histochrome in combination with Gingko Biloba or diquertin stabilizes the antioxidant potential of tear fluid. Combined treatment with histochrome, Gingko Biloba, and diquertin produced a strong stabilizing effect on the antioxidant potential of tear fluid and blood plasma and concentration of MDA. This effect can be explained by polyfunctionality of antioxidants. These substances potentiate the effects of each other on free radical oxidation and LPO.

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