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Antioxidant status of dog aqueous humor after extracapsular lens extraction

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Abstract

We determined the antioxidant status of the aqueous humor after extracapsular lens extraction in 14 mongrel dogs weighing about 10 kg. The animals were examined by slit lamp biomicroscopy, applanation tonometry and indirect ophthalmoscopy. One eye was submitted to conventional extracapsular lens extraction and the other was used as control. Samples of aqueous humor were obtained by anterior chamber paracentesis before and at days 1, 2, 3, 7 and 15 after surgery. Total antioxidant status was determined as the capacity of aqueous humor to inhibit free radical generation by 2,2-azobis(2-amidopropane) chlorine. Ascorbic acid concentration was measured by HPLC with UV detection. Protein content was determined with the biuret reagent. Statistical analysis was performed by ANOVA followed by the Tukey-Kramer test. Protein concentration increased from 0.61 to 22 mg/ml 24 h after surgery. These levels were maintained and returned to normal at day 7. Total antioxidant capacity was reduced from 50 to about 30 min until day 3 and at day 7 it was equal to control. Ascorbic acid levels were reduced from 252 to about 110 μM and then returned to control values at day 15. Considering the importance of ascorbic acid concentration in aqueous humor for the maintenance of the antioxidant status of the anterior segment of the eye, the decrease of antioxidant defenses suggests that the surgical procedures promote an oxidative stress condition in the eye.

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- Extracapsular lens extraction

The increase in free radical or reactive oxygen species (ROS) production or the decrease in antioxidant mechanisms generates a condition called oxidative stress, defined as the imbalance between pro- and antioxidants in favor of the oxidants (1). The eye is exposed to high ROS concentrations both under normal conditions, e.g., singlet oxygen produced by UV radiation, or in pathological conditions such as uveitis (2,3). For protection against oxidative damage the eye is equipped with both enzymatic and nonenzymatic antioxidants. Among nonenzymatic antioxidants, ascorbic acid concentration is unusually high when compared with other tissues. These levels have been reported to be 20-fold higher in humans (4) and 9-fold higher in mongrel dogs when

compared to plasma (5). Diurnal animals present much higher levels of ascorbic acid in the aqueous humor than nocturnal ones, suggesting a protective role of the acid against eye tissue photo-oxidation (4). This high concentration is maintained in aqueous humor by an active uptake of ascorbic acid by the iris and ciliary body (6). Although the mechanism is still unclear, some investigators suggest a role for ascorbic acid in the protection of the eye against ROS (4). Giblin et al. (7) demonstrated a direct correlation between ascorbic acid and H_2O_2 levels in the aqueous humor.

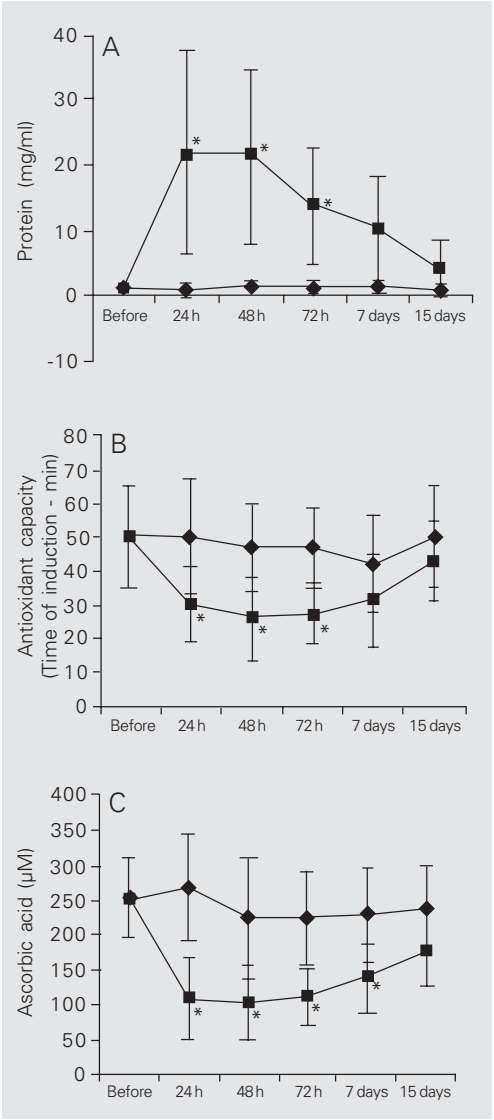

Figure 1. Effect of extracapsular lens extraction on protein content (A), total antioxidant capacity (B) and ascorbic acid concentration (C) of the aqueous humor. Protein is reported as mg/ml, antioxidant capacity as time of induction in minutes, and ascorbic acid in µM. *P < 0.05 compared to the value before surgery (ANOVA followed by the Tukey-Kramer test). Control (lozenges, N = 14); operated (squares, N = 14).

After surgery or paracentesis or in the presence of uveitis, a blood-aqueous barrier breakdown occurs, with increased amounts of protein and cells in the aqueous humor (8). These cells are responsible for increased production of ROS in the aqueous humor with consequent tissue damage (2). Another factor that can contribute to the increase of the oxidative condition of aqueous humor is the alteration of the iris-ciliary body that follows surgery, with decreased production of aqueous humor (9).

The objective of the present study was to assess the antioxidant status of aqueous humor after extracapsular lens extraction in dogs.

The study was conducted on 14 mongrel dogs weighing about 10 kg. Animals were examined by slit lamp biomicroscopy, applanation tonometry (Tonopen, BioRad Inc., Santa Ana, CA, USA) and indirect ophthalmoscopy to assure that the eyes were healthy. One eye was submitted to conventional extracapsular lens extraction. Under general inhalation anesthesia, a 170º clear cornea incision was made, the anterior lens capsule removed, the lens gently pushed out of the wound using a Wilder lens loop, the anterior chamber was irrigated with balanced salt solution to remove all additional lens material, and the corneal incision was closed with simple interrupted 9-0 mononylon sutures (10). The other eye was used as control. Samples of aqueous humor (0.2 ml) from both eyes were obtained by anterior chamber paracentesis before and at days 1, 2, 3, 7 and 15 after surgery. No topical or systemic medication was used. The procedures was carried out according to the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research (11).

Total antioxidant activity in the aqueous humor was considered to be the capacity to inhibit free radical generation by an aqueous solution of 2,2-azobis(2-amidopropane) chlorine (ABAP). An aqueous solution of ABAP produces peroxyl radicals that are scavenged

by antioxidants of the aqueous humor. Chemiluminescence emitted by peroxyl radicals was measured with a scintillation counter. The amount of antioxidants in aqueous humor depends on the time for light emission recovery, reported as minutes (12). Ascorbic acid was determined using an isocratic high performance liquid chromatography system with UV detection (254 nm) after acid extraction (10% metaphosphoric acid). A C18 reverse phase column with a 2% metaphosphoric acid mobile phase was employed (13). Protein content was determined by the method of Lowry et al. (14). Statistical analysis was performed by ANOVA followed by the Tukey-Kramer test.

The postoperative period was uneventful with no vitreous loss or posterior capsule rupture. To determine the time course of the parameters, each time value was compared with the value obtained before surgery. Protein concentration in aqueous humor increased 35-fold 24 h after surgery. These high levels were maintained over the subsequent days and returned to normal at day 7 (Figure 1A). Total antioxidant capacity was reduced by 40% until day 3 and was statistically equal to the control eye at day 7 (Figure 1B). Ascorbic acid levels showed a similar pattern but only returned to control values at day 15 (Figure 1C).

Paracentesis *per se* (control group) did not modify the parameters measured except for a slight nonsignificant increase in protein content. The antioxidant status of biological fluids is fundamental for preventing damage by oxidant species. A simple and predictable way to measure this condition is to assess the capacity of a biological fluid to inhibit free radical generation by an aqueous solution of ABAP. A similar method was used by other investigators to assess the total antioxidant activity of aqueous humor, with predictable responses (15). These investigators showed that the kinetics of aqueous humor was comparable to that of ascorbic acid, the main antioxidant component of aqueous humor, by inhibiting the formation of a colored radical cation produced by the action of metamyoglobin and hydrogen peroxide on 2,2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid).

The present study demonstrates that after extracapsular lens extraction the total antioxidant capacity of aqueous humor was decreased by 40%, indicating an oxidative condition of the eye that paralleled the decrease in ascorbic acid concentration. A decreased concentration of ascorbic acid in aqueous humor has been demonstrated in human patients with idiopathic acute anterior uveitis (16) and in experimental endotoxin-induced ocular inflammation in rabbits (17). Our findings can be explained in part by the inflammation that follows the surgical procedure, confirmed by the presence of increased amounts of protein in the aqueous humor (Figure 1A). The decreased antioxidant capacity of aqueous humor can contribute to an oxidative condition of the anterior chamber of the eye. This could increase the chances of injury by oxygen species locally produced by inflammatory cells in corneal and other structures bathed by the aqueous humor.

Another substance that may contribute to the antioxidant activity of aqueous humor is uric acid. In diurnal birds such as chickens and turkeys, uric acid levels are much higher than ascorbic acid levels, playing the role of a UV filter as done by ascorbic acid in mammals. In mammals the concentration of uric acid in aqueous humor is low and contributes very little to total antioxidant capacity (18).

The oxidative stress that occurs after extracapsular lens extraction suggests that all care should be taken during surgical procedures to minimize the inflammatory process and the consequent imbalance of antioxidants in the aqueous humor. More studies should be conducted in order to control this pathological state frequently observed in the eye clinic.

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