Original Article

Effect of air bubble on inflammation after cataract surgery in rabbit eyes

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Purpose: Intense inflammation after cataract surgery can cause cystoid macular edema, posterior synechia and posterior capsule opacification. This experimental study was performed to investigate the effect of air bubble on inflammation when given to anterior chamber of rabbit eyes after cataract surgery. **Materials and Methods:** 30 eyes of 15 rabbits were enrolled in the study. One of the two eyes was in the study group and the other eye was in the control group. After surgery air bubble was given to the anterior chamber of the study group eye and balanced salt solution (BSS; Alcon) was left in the anterior chamber of control eye. **Results:** On the first, second, fourth and fifth days, anterior chamber inflammations of the eyes were examined by biomicroscopy. On the sixth day anterior chamber fluid samples were taken for evaluation of nitric oxide levels as an inflammation marker. When the two groups were compared, in the air bubble group there was statistically less inflammation was seen. (1, 2, 4. days P = 0,001, and 5. day P = 0,009). **Conclusions:** These results have shown that when air bubble is left in anterior chamber of rabbits' eyes after cataract surgery, it reduced inflammation. We believe that, air bubble in the anterior chamber may be more beneficial in the cataract surgery of especially pediatric age group, uveitis patients and diabetics where we see higher inflammation. However, greater and long termed experimental and clinical studies are necessary for more accurate findings.

Key words: Air, cataract surgery, inflammation, rabbit

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Cataract surgery is one of the most frequently performed surgeries in the world today. Although modern cataract surgery is a very safe and predictable procedure, in some special cases it is usually associated with varying degrees of postoperative inflammation.[1] The composition of aqueous humor is dependent mainly on the integrity of blood-aqueous barrier. A disruption in the blood-aqueous barrier may result in the production of chemical inflammatory mediators such as oxygen-free radicals, cyclooxygenase and lipooxygenase metabolites of arachidonic acid within the aqueous cavity and leads to clinically detectable perilimbal injection, flare and cells in the anterior chamber.[2,3] In recent years the operative technique in cataract surgery has improved and the operation has become less traumatic to the eye.[4] Nonetheless, some patients still exhibit clinically significant postoperative ocular inflammation after cataract surgery. Left untreated, this inflammation can interfere with the patient's visual rehabilitation or lead to further complications, such as pain and discomfort acutely, or cystoid macular edema (CME) chronically.[5-7]

Clinically, slit-lamp biomicroscopy is the gold standard to evaluate anterior chamber inflammation.[8] In 1959, Hogan *et al*. established a grading system to assess anterior chamber flare and cells.[9]

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The role of the cytokines in wound healing and inflammation is currently under investigation. Nitric oxide (NO), a noxious free- radical gas and biochemical material that is of vital physiological significance, can readily diffuse through cell membranes to induce several biological actions in mammals. Any immunologic or inflammatory stimuli induce the production of NO by the expression of the inducible isoform of the nitric oxide synthase (NOS).[10] Previous experimental and human studies have showed that NO and cytokines, including IL-1 β, IL-2R, IL-6, and TNF-α, play a crucial role in inflammatory and immunologic response.[11,12]

The purpose of this study was to determine whether air bubble left in anterior chamber of the rabbit eyes after cataract extraction has protective effect on blood aqueous barrier as measured clinically by using slit lamp biomicroscopy and biochemically by aqueous nitrous oxide levels.

Materials and Methods

Animals and anesthesia

This study included 30 eyes of 15 adult New Zealand White rabbits weighing between 2,0-3,0 kg. Animals were preoperatively screened to preclude those animals for preexisting ocular pathology. One of the two eyes was in the study group and the other eye was in the control group in a random method.

For all procedures, anesthesia was induced with intramuscular injection of ketamine HCL (5 mg/kg) and xylazine HCL (2 mg/kg). Pupils were dilated with 1-2 drops each of 2.5% phenylephrine HCl (Mydfrin; Alcon) and 1% tropicamide HCl (Tropamid; Bilim). Topical application of proparacaine hydrochloride 0.5% (Alcaine; Alcon) was also used for local anesthesia. All procedures were approved by the Animal Care Committee of the University of Kocaeli-Turkey, and all adhered to the ARVO Statement for the Use of Animals

in Ophthalmic and Vision Research.

Surgical procedure for rabbit lensectomy

Under sterile conditions, the lensectomy procedure was performed by the same surgeon in either eye with endocapsular phacoemulsification with an anterior chamber maintainer and a corneal incision similar to that used in a standard cataract surgery performed routinely in our clinic. Following general anesthesia, the animal was draped, a speculum was inserted, an anterior chamber maintainer is positioned at 2 o clock, and from a sideport temporally a continious curvilinear capsularhexis was implemented with a push-pull cystotome. From a 3.0 mm superior incision ultrasonic fragmentation and aspiration of the lens is done with a phacomachine.(Surgical Design;USA). [Figs. 1 and 2] At the end of the procedure, anterior chamber of the study group eyes formed with an air bubble occupying 2/3 of the anterior chamber [Fig. 3] and the other eye was formed with balanced salt solution (BSS; Alcon). Corneal tunnel incisions were secured with a 10/0 nylon suture.(Alcon)

Clinical scores

The rabbits were examined on days 1,2,4 and 5 with a slit lamp biomicroscope (Topcon SL 7F;Japan) for flare, corneal edema and for the amount of air bubble in anterior chamber. Postoperative inflammation was graded at defined intervals over a five day period according to Hogan anterior chamber flare classification system and graded from 0 (normal) to 4 (severe), using the lens scatter as a reference for grade 4.

On the 6th postoperative day under sterile conditions and general anesthesia; with a insulin syringe anterior chamber paracentesis is performed for aqueous humor sample for calculation of NO amount in anterior chamber.

From this sample nitrite amount was determined with the spectrophotometric Griess reaction.[13] First the aqueous humor sample 100 µl is added to mixture of 400 µl of distilled water and 300 µl of 0.3 N NaOH solution. The solution is kept for 5 min at room temperature; then 300 µl of 5% (w/v) $ZnSO_4$ is added. The mixture is allowed to stand for another 5 min after shaking and is then centrifuged at 10 000 g for 20 min at 4°C. The total of NO-metabolites nitrate (after reduction to nitrite by cadmium granules) and nitrite was assayed colorimetrically using the Griess reaction.

Statistical methods

SPSS (Statistical Package for Social Sciences) for Windows 10.0 (SPSS inc.) was used for statistical analysis. A *P* value less than 0.05 was considered statistically significant for all analyses. For comparison of quantitative data Mann Whitney U test was used.

Results

There were no preexisting ocular pathology in any of the rabbit eyes. Biomicroscopically there were no postoperative corneal edema or increase in corneal thickness in any of the eyes. In postoperative day 1 air bubble was filling only 1/3 of the anterior chamber, and no air bubble was left on day 4.

One day postoperatively, flare level was very severe in the control group. Average flare level was lower (0,80 ± 0,43) in air bubble group [Table 1] [Figs. 4 and 5] then in the control group (irrigation solution) (4.00 ± 0.0). [Fig. 6]. The difference was statistically significant between air bubble group and control group ($P < 0.01$) Second day postoperatively, flare level was again lower in air bubble group (0.33 ± 0.48) than the control group (3.26 ± 0.45) ($P < 0.01$), fourth day post operatively flare level was again lower (0.33 ± 0.48) than the control group (2.80 ± 0.67) ($P < 0.01$) 5th day post operatively there was no sign of flare in the air bubble group (0.00 ± 0.00) but although very low there was still statistically significant flare in the control group (1.06 ± 1.03) ($P < 0.01$) [Table 1].

Nitric oxide levels

NO levels in the air bubble group was (15.00 ± 5.26) µM / L and (31.33 ± 14.45) µM / L in the control group with $P < 0.010$ statistically significantly lower in the air bubble group, in accordance with the biomicroscopic values [Table 2] [Fig. 7].

Discussion

The primary objective of this study is to compare the effect of air bubble on anterior chamber inflammation after cataract surgery. Recent advances in cataract surgery such as new phacoemulsification machines, viscoelastic agents and new

Table 1: Statistical analysis of biomicroscopic scores between two groups according to Hogan flare scoring system

Biomicroscopic examination	Air bubble (*n* = 15)		BSS (*n* = 15)		*P*
	Median	Avg. ± S.D.	Median	Avg. ± S.D	
Postoperative 1. Day	1	0.80 ± 0.41	4	4.00 ± 0.0	0.001**
Postoperative 2. Day	0	0.33 ± 0.48	3	3.26 ± 0.45	0.001**
Postoperative 4. Day	0	0.33 ± 0.48	3	2.80 ± 0.67	0.001**
Postoperative 5. Day	0	0.00 ± 0.00	1	1.06 ± 1.03	0.009**

**$P < 0.01$ statistically significant

Table 2: Statistical analysis of comparison of nitric oxide levels between two groups

	Air bubble group (*n* = 15)		BSS (*n* = 15)		*P*
	Median	Avg. ± S.D.	Median	Avg. ± S.D	
Nitric oxide level	17.5	15.00 ± 5.26	27.5	31.33 ± 14.45	0.010*

*$P < 0.05$ statistical significance

intraocular lens designs has reduced the extent of ocular injury, but not completely eliminated the production and release of inflammatory mediators, breakdown of blood aqueous barrier (BAB). BAB damage can also be seen even after non

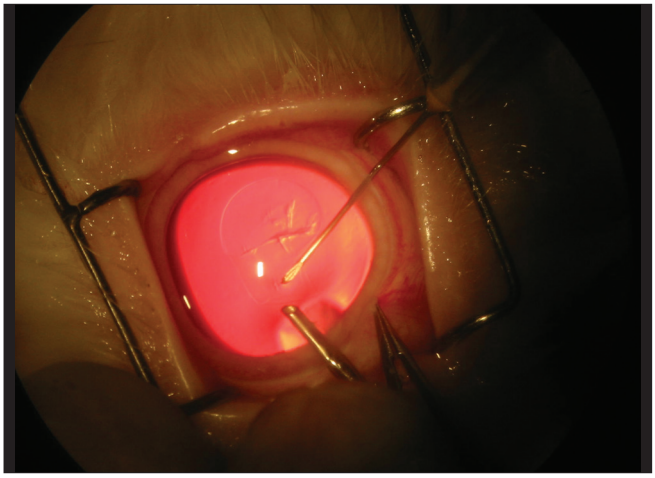

Figure 1: Capsularhexis of rabbit eye under ACM

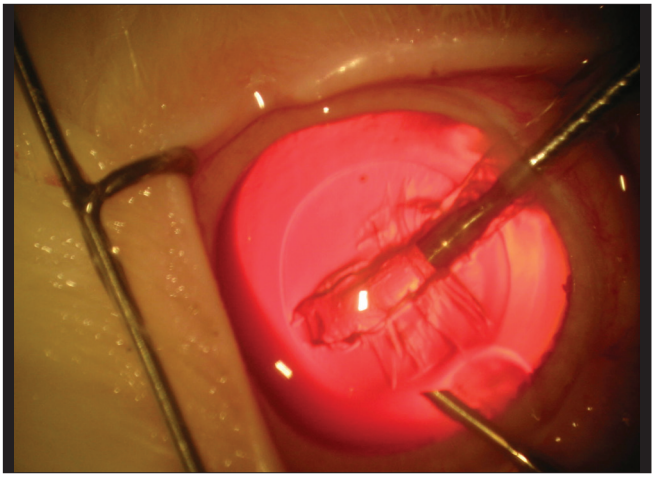

Figure 2: Phacoemulsification of rabbit eye under ACM

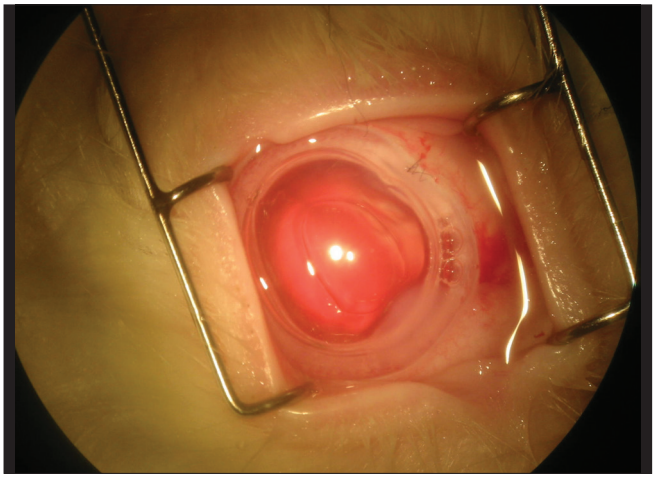

Figure 3: Air bubble in anterior chamber after cataract surgery of rabbit eye

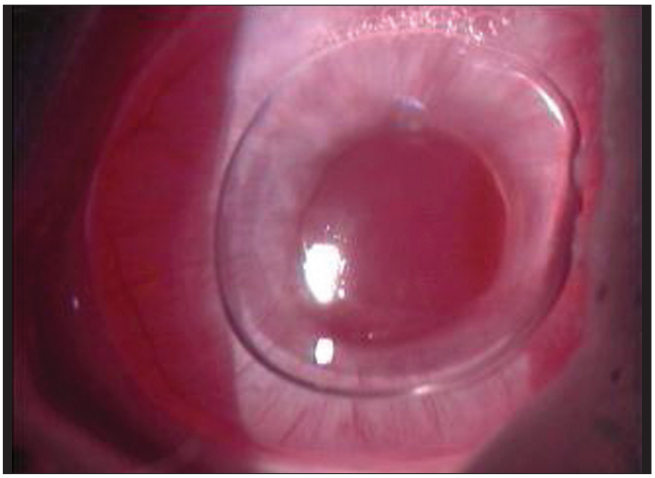

Figure 4: Anterior chamber of rabbit eye in the study group

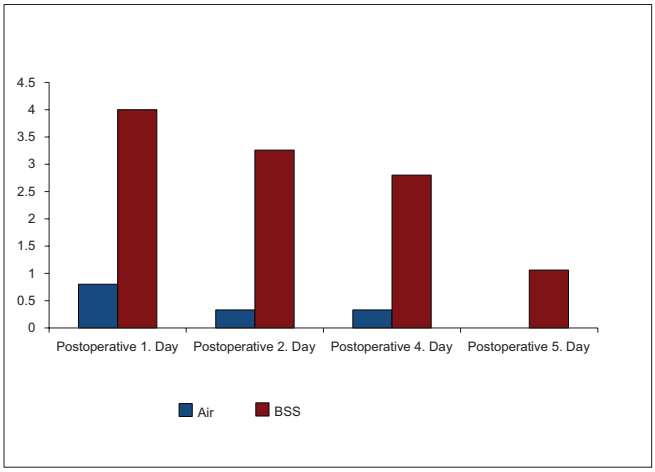

Figure 5: Graphical representation of daily scored biomicroscopic flare values

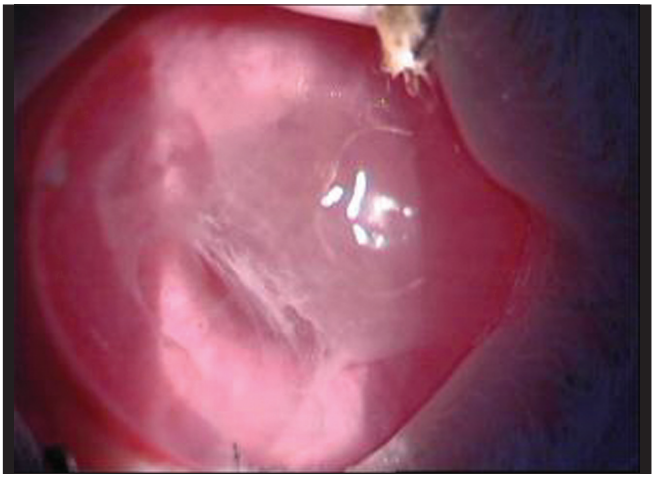

Figure 6: Flare in anterior chamber of the other eye of the same rabbit

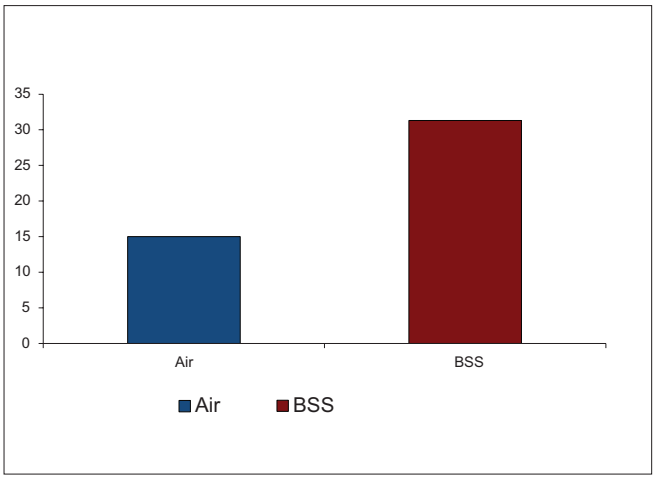

Figure 7: Graphical representation of average nitric oxide values between groups

complicated ocular surgeries. Especially, diabetes, pediatric age group, history of previous intraocular surgery, glaucoma, uveitis and pseudoexfoliation are the main risk factors for severe inflammation.[14-17] Especially, after congenital cataract surgery complication rates are increased. Probably most important factor contributing to the high complication rate is the patient's age at the time of the surgery. Keech *et al*. found that there is significant increase in the number of complications in patients operated upon in 2 months of age as compared with children operated on after that age. One of the factors may be the marked inflammation and scarring that often occurs after surgery in an immature eye.[18]

Any traumatic events in the eye, whether due to injury or surgery, results in a various extents of disturbance on the BAB. An increase of the protein concentration in the aqueous is the most straigt forward evidence for the breakdown of BAB. Limited by the available examination technique, the protein concentration in the anterior chamber can only be estimated by slit-lamp microscopy.[8,19]

In this study postoperative inflammation was evaluated by clinical biomicroscopy and aqueous humor NO levels. The laser cell flare meter is currently the most accurate and sensitive method of measuring anterior chamber flare and cells, which directly reflects the BAB damage.[20] There is close agreement in measurements between biomicroscopy and LFCM which has been well documented in the literature, but also its availability is very limited in the ophthalmology cilinics especially due to cost effectiveness.[21,22] Several authors compared air bubble and viscoelastic agents in production of corneal edema and sterile endopthalmitis and found higher incidence of these two complications when the surgeons have used viscoelastic agents instead of air bubble.[23,24] Attarzadeh H. has recommended ophthalmic surgeons to use air bubble instead of viscoelastic agents during intraocular lens (IOL) implantation to decrease posterior capsule opacification and pigment deposition over IOL.[25] In the present study, in all examinations the group in which air bubble is given there was significantly very low inflammation where as the ones in which balanced salt solution used there was significantly very severe inflammation make us think that air bubble can have an anti inflammatory effect by decreasing BAB disruption which is maybe the reason for results of these previous studies.

Excessive disruption of the BAB following cataract surgery delays visual recovery and is associated with the need for prolonged medication. It also potentially leads to many ocular complications such as glaucoma, cystoid macula oedema, posterior synechia, and posterior capsular opacification.[20,26] Eyes that are predisposed to postoperative inflammatory reactions, such as in pseudoexfoliation syndrome, glaucoma or diabetes, can be expected to benefit even more from the reduced surgical trauma induced by the phacoemulsification technique.[27,28]

A reduction in postoperative inflammation is associated with less patient discomfort and reduced requirements for topical medication, faster visual rehabilitation, and potentially a reduction in postoperative complications such as macular edema and posterior capsule opacification. It is, therefore, important to minimise surgical trauma to the BAB.[20,26]

Although corticosteroids are the drug of choice in treatment of inflammation; the serious side effects of these drugs such as glaucoma, delayed wound healing, corneal ulcer, etc.,have forced ophthalmologists to find safer treatment modalities.[29,30] Mansour *et al*. have studied anti- inflammatory effects of air bubble given into vitreus cavity of endophthalmic rabbit eyes. As a result clinically and histopathologically, gas filled eyes demonstrated less inflammation (cornea, anterior chamber, and vitreous) than did vitrectomized eyes without gas. The reason for less inflammation was thought as the large gas bubble reduces the volume of vitreous substance, which serves as a growth medium for microorganisms and is relatively poorly penetrated by several systemic antibiotics.[31] But we also have to mention that large air bubble can lead to pupil block glaucoma, to prevent this complication while adjusting amount of air bubble, proper position should be given to the patient.

Mishra *et al*. reported that after performing small incision cataract surgery (Non-Phaco SICS) at the end of the surgery, after aspiration of the viscoelastic they have formed the anterior chamber with air and BSS and concluded that they routinely reform the anterior chamber with air bubble and BSS at end of the cataract surgery in children to make it suture less.[32] Sim *et al*. also reported that if air bubble is left after sutureless cataract surgery; air bubble, which is compressible, allows the anterior chamber more compliance This prevents wound leak and suction/inflow with positive and negative pressures created by external forces. This is especially crucial during the first 24 h after intraocular surgery. So that this simple procedure may reduce intraocular contamination and rate of postoperative endophthalmitis.[33] Several studies have been performed to examine the toxic effects of air and various long-lasting gases, such as sulfur hexafluoride (SF6), perfluoropropane (C3F8), and octafluorocyclobutane in the anterior chamber of animals and humans.[34-37] Air is commonly used to re-form the anterior chamber in a variety of surgical procedures.[34] Norn *et al*. studied the effect of air on the human corneal endothelium by reestablishing the anterior chamber with air after cataract extraction. There were no adverse effects six months later.[38] Air has repeatedly been reported to damage the corneal endothelium after introduction to the anterior chamber experimentally or clinically by anterior segment surgery.[33,37] The damage depends on the extensiveness of aqueous washout. Gaseous interface with the corneal endothelial cells is unnatural

and probably causes some damage to the cells; however, air appeared to have no long term deleterious effects.[39,40] Also when the gas bubbles are able to move around in the aqueous or vitreous cavities, evidence of toxicity is not seen clinically. This previously observed fact is also true for perfluoropentane, even though it persists for months.[41]

We have obtained samples from anterior chamber for NO as inflammation marker. It has been demonstrated that NO plays an important role in regulating the local blood flow and aqueous outflow facility.[11,42] It has also been emphasized that changes in vascular hemodynamics in anterior uveitis correlates with increased levels of NO in the aqueous humor.[9] Previous studies have shown that paracentesis did not alter the protein concentration or composition significantly.[43,44] Clinical studies suggest that aqueous flare and cell intensity after cataract surgery in the early postoperative period is highest on days 1 and 3.[45] Jurowski *et al*. have assessed the highest levels of NO within the first 3 postoperative days. As infiltrating inflammatory cells induce NO production, NO is thought to be associated with some pathologic conditions involving these cell types. These findings may suggest that BAB breakdown after cataract surgery is a combination of different factors and NO may be partly responsible for this event.[46] If we affirm that the NO level in aqueous humor may reflect, in part, postsurgery trauma and since NO is an important factor potentially contributing to BAB breakdown, the identification of basic mechanisms associated with postoperative inflammatory changes would affect future treatment strategies.

In our study air bubble group had lower NO levels as compared with the control group. It was statistically significant that air bubble group has lower inflammation on the 6th day postoperatively. These results were similar to the biomicroscopic examination results between the two groups that make us think that, maybe the anti inflammatory effect of air bubble could have been by decreasing NOS production by an unknown mechanism which has to be re-examined with other studies.

In conclusion, to the best of our knowledge, this is the first study to evaluate air bubble effect on inflammation in rabbit eyes after cataract surgery. These results have shown that when air bubble is left in the anterior chamber of rabbit eyes after cataract surgery, it reduces inflammation. We believe that, air bubble in the anterior chamber may be more beneficial in the cataract surgery in especially pediatric age goup, uveitis patients and diabetics where we see higher inflammation. However, greater and long termed experimental and clinical studies are necessary for more accurate findings.

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