Antioxidant Protection against Corneal Damage by Free Radicals during Phacoemulsification

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Purpose. To examine the role of ascorbic acid in reducing corneal endothelial cell loss secondary to high-energy ultrasound energy during phacoemulsification surgery.

Methods. Seventeen rabbit eyes were subjected to prolonged phacoemulsification within the anterior chamber, without manipulation or damage to other ocular structures. In nine eyes, a balanced salt ophthalmic solution was used as the phacoemulsification irrigation solution, and in eight eyes the solution plus 0.001 M ascorbic acid was used, all other parameters being identical between the two groups. Specular microscopy was performed in all eyes before and 1 week after surgery. The animals were then killed, and the corneas were examined histologically.

Results. There was no significant difference in preoperative endothelial cell counts between the two groups. Postoperative cell counts were reduced by 453.9 ± 233.3 (SEM) cells/mm^2 in the solution-alone group versus 123.2 ± 196.4 (SEM) cells/mm^2 in the solution-plus-ascorbic acid group, ($P = 0.011$). Corneal histology revealed a marked difference in endothelial cell morphology between the two groups.

Conclusions. The addition of ascorbic acid to the irrigation solution significantly reduced the amount of endothelial cell loss during phacoemulsification by approximately 70%. This is thought to be due to the free-radical–scavenging properties of ascorbic acid. Further studies are warranted to find the optimal concentrations and combinations of free radical scavengers to be used in phacoemulsification irrigation solutions. (*Invest Ophthalmol Vis Sci.* 2003;44:1866–1870) DOI:10.1167/iovs.02-0892

Since the introduction of high-energy ultrasonic medical devices, increasing interest and concern have been directed toward the biological and cellular effects of the production of large amounts of free radicals and ultraviolet radiation within tissues.[1–10]

Studies of the harmful effects of phacoemulsification on corneal endothelial cells suggest that much of this damage is mediated by free radicals.[10–13] Subsequent articles have emphasized the potential protective effects of various free radical scavengers,[10,14–18] and suggest that these substances may help prevent phacoemulsification-induced endothelial cell loss, a common and well-documented side effect of cataract surgery.[19–25]

Holst et al.[7] showed the formation of large amounts of free radicals and ultraviolet radiation in phacoemulsification in both in vivo and in vitro environments. This, it was shown, could be reduced by the addition of superoxide dismutase to the irrigating solution; however, they did not examine whether this reduction could effectively reduce endothelial cell loss.

Other studies[14,17] have shown a nonspecific beneficial effect of irrigating solutions containing glutathione (a free radical scavenger) in reducing cell loss from prolonged endothelial exposure to irrigating solutions, but we did not specifically investigate the effects of phacoemulsification or free radical damage. In a study in which a similar irrigation model in dogs was used,[26] the investigators found no beneficial effect of glutathione. A study of the protective effects of different viscoelastic materials against endothelial damage by intraocular hydrogen-peroxide solutions[15] suggested that their protective effect may be due in part to free-radical–scavenging properties.

Several researchers have compared the results of cataract surgery using a balanced saline ophthalmic irrigating solution to those obtained using solutions containing glutathione. Some have reported a protective effect of glutathione on the corneal endothelium,[27,28] whereas others have found no effect.[29] These studies, while important in determining the clinical use of glutathione, do not exclude other causes of endothelial damage, such as intraocular lens implantation, release of inflammatory substances in the eye, or release of lens particles. Also, these studies examined only one concentration of one free radical scavenger (glutathione), because it is the only commercially marketed solution in this category. We elected to examine the role of ascorbic acid, a well-known scavenger of free radicals,[30–32] because this chemical is found in naturally high concentrations in normal aqueous,[33,34] would therefore be less likely to cause chemical damage to intraocular structures, and has not previously been tested in the present setting to our knowledge.

To elucidate the role of free radicals in endothelial cell loss secondary to phacoemulsification, we devised an experiment that would attempt to isolate the endothelial damage due to formation of free radicals during phacoemulsification from mechanical and thermal damaging effects.

Methods

Seventeen eyes of 17 New Zealand White female rabbits, weighing 3 to 4 kg each, were exposed to high-energy ultrasound by the activation of a phacoemulsification probe in the anterior chamber. Treatment and handling of the rabbits was performed in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. Before surgery, the rabbits were anesthetized by intramuscular injection of 35 mg/kg ketamine hydrochloride and 7 mg/kg xylazine, and

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TABLE 1. Specular Microscopy Results

Eye	Irrigating Solution	Preoperative Cell Count/mm^3	Postoperative Cell Count/mm^3	Cell Loss/mm^3	% Change in Endothelial Cell Density
1	Solution*	3011	2381	630	−20.9
2	Solution	3294	3027	267	−8.1
3	Solution	3000	2390	610	−20.3
4	Solution	3631	3112	519	−14.3
5	Solution	3058	2205	853	−27.9
6	Solution	3199	2908	291	−9.1
7	Solution	3123	2914	209	−6.7
8	Solution	3135	2883	252	−8.0
9	Solution+AA†	3058	3195	−137	+4.7
10	Solution+AA	2756	2835	−79	+2.8
11	Solution+AA	3021	3170	−149	+4.9
12	Solution+AA	2885	2655	230	−7.9
13	Solution+AA	3493	3363	130	−3.7
14	Solution+AA	3525	3154	371	−10.5
15	Solution+AA	2968	2765	203	−6.8
16	Solution+AA	3082	2854	228	−7.4
17	Solution+AA	3137	2825	312	−9.9

The statistical test revealed no differences between the two groups in preoperative cell counts at the 5% significance level (Mann-Whitney test $P = 0.37$).

* Balanced solution.

† Balanced solution with ascorbic acid.

the right eye was then examined by specular microscopy (EM-1000 specular microscope; Tomey, Waltham, MA), obtaining three corneal endothelial photographs for analysis, all from within the central 3 mm of cornea. The right eye was cleansed with topical povidone iodine and draped, and a lid speculum was inserted. Next, a clear corneal incision was made in the superotemporal corneal quadrant with a disposable 3.2-mm keratome blade. Using 70% power, and 25 mL/min of irrigation, a 20-g phacoemulsification probe (Series Ten Thousand Phacoemulsification System; Alcon Surgical, Fort Worth, TX) was introduced through the corneal incision into the anterior chamber, taking care to avoid touching all ocular structures including the lens and cornea, and activated in the center of the anterior chamber. Each eye was continuously irrigated, with the power turned alternately on and off every 10 seconds to avoid overheating, until the required 5 minutes of net time (10 minutes in all) was completed.

The rabbits were randomized to receive either balanced salt solution, or BSS with 0.001 M of sterile ascorbic acid introduced into the BSS bottle immediately before phacoemulsification. The two groups were similar in all other respects of surgical and postoperative treatment. Each animal was number encoded before treatment, and surgery, postoperative, and histologic examinations and analysis were all performed with blinding as to the treatment grouping.

On completion of phacoemulsification, the incision was sealed by injection of BSS solution (for both groups) into the incision margins, followed by a subconjunctival injection of betamethasone acetate and betamethasone phosphate (Celestone Chronodase; Schering-Plough, Kenilworth, NJ) and gentamicin. No additional postoperative medications were given.

One week later, the rabbits were again anesthetized as described above, the surgical eye was again examined by specular microscopy, and the rabbits were killed by an overdose of phenobarbital. The right eye of each rabbit was enucleated and preserved in formaldehyde, and corneal sections were stained with hematoxylin and eosin and examined by an experienced ocular pathologist who was blinded to the treatment received by each rabbit, as mentioned previously.

The specular microscopy results were analyzed with the software application for endothelial cell analysis that accompanied the specular microscope (EM-1100 software; Tomey). The results of the three preoperative and three postoperative endothelial cell counts were averaged separately, and the reduction in cell count 1 week after surgery was calculated by subtraction. The nonparametric Mann-Whitney test was used to examine possible differences in preoperative cell counts between the two groups, as well as differences in postoperative cell count reduction. Cell counts are presented as the mean ± SEM.

RESULTS

Preoperative and postoperative endothelial cell counts are shown in Table 1, in random and nonchronological order within each group. It should be noted that we previously found the normal interexamination variation between endothelial cell counts to be approximately ±5%, and postoperative results showing minor increases in endothelial cell counts are probably due to this variation.

The reduction in endothelial cell count (cells per square millimeter) 1 week after surgery was 453.9 ± 233.3 (SEM) in the group that received the irrigation solution alone and 123.2 ± 196.4 (SEM) in the group treated with the solution plus ascorbic acid (Fig. 1).

The postoperative reduction in cell count differed significantly between the two groups (Mann-Whitney test $P = 0.011$). Thus, the cell count was significantly higher in the group treated with solution plus ascorbic acid than in the group treated with solution alone. The mean reduction in cell count in the solution-alone group was more than 3.5 times greater than in the group treated with solution plus ascorbic acid.

Histologic slides prepared from all corneas of rabbits in the two treatment groups showed marked differences in endothelial cell morphology. Endothelial cells from the central corneas in the solution-alone group contained more and much larger vacuoles than those from the group treated with solution plus ascorbic acid (Fig. 2). For each group, similar findings were obtained in all 10 central corneal sections examined.

DISCUSSION

In previous studies, our group has examined the production of free radicals by ophthalmic (and other medical) phacoemulsi-

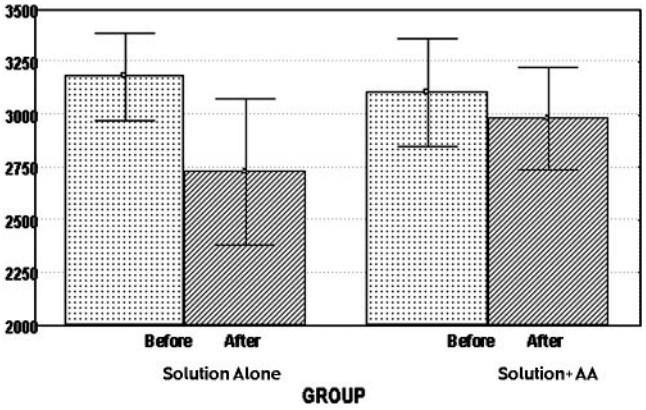

FIGURE 1. Preoperative and postoperative endothelial cell counts in groups treated with a saline ophthalmic irrigation solution (Solution Alone) and with the same solution plus ascorbic acid (Solution+AA).

fication probes in vitro, as well as the effects of free radical scavengers, such as ascorbic acid and glutathione, on the formation of these radicals.[8,10,35] As a continuation of those studies, we designed the present in vivo experiments with the purpose of determining whether our chemically quantified results could be translated into clinically significant protective effects on the corneal endothelium.

This study was preceded by a pilot study in which we used smaller groups of rabbits to establish optimal parameters, especially with regard to various amounts of phacoemulsification time. We found that phacoemulsification times of less than 5 minutes produced minor endothelial cell loss, indistinguishable from the normal inter-examination variation in specular microscopy endothelial cell count analysis (±5%). Also, as can be seen in Table 1, the variation in endothelial cell loss within each treatment group was substantial and only the large protective effect of the ascorbic acid enabled us to show a statistically significant difference between the two groups, despite this variability. In contrast, phacoemulsification times significantly longer than 5 minutes caused severe endothelial cell loss, with resultant corneal edema that prevented postoperative specular microscopy. We therefore decided that 5 minutes of exposure to anterior chamber phacoemulsification would cause sufficient and measurable endothelial damage. As this is longer than most clinical phacoemulsification times, we believe the protective effect of scavengers in phacoemulsification surgery may be more pronounced in difficult and prolonged surgeries. Also, as rabbit endothelium is capable of some regeneration, it was decided that a period of 1 week between surgery and analysis would enable us to measure the extent of endothelial cell damage while minimizing the effects of regeneration on cell counts.[37–40]

The eyes we studied were not subjected to cataract surgery, because this would have introduced several confounding and nonquantifiable causes of endothelial damage, such as the intraocular release of lens particles, various amounts of remaining intracapsular cells and debris, and different degrees of ocular inflammation and release of inflammatory products. Also, by restricting surgery to a minimum we were able to minimize variations in surgical technique between eyes. In terms of free radical production, phacoemulsification performed in the anterior chamber is sufficient to release the radicals under study, while avoiding the mentioned confounding effects of cataract surgery.

Several previous studies regarding endothelial cell regeneration and repair have shown that 1 week of postoperative healing in rabbits is comparable to approximately 3 months in humans and that, after this time, cellular regeneration (not found in humans) takes place,[38,40] which may be a confounding factor, altering postoperative cell counts. Indeed, several studies have provided evidence that ascorbic acid may be an important factor in endothelial cell healing, migration, and regeneration.[41,42] We therefore decided to kill the animals 1 week after phacoemulsification.

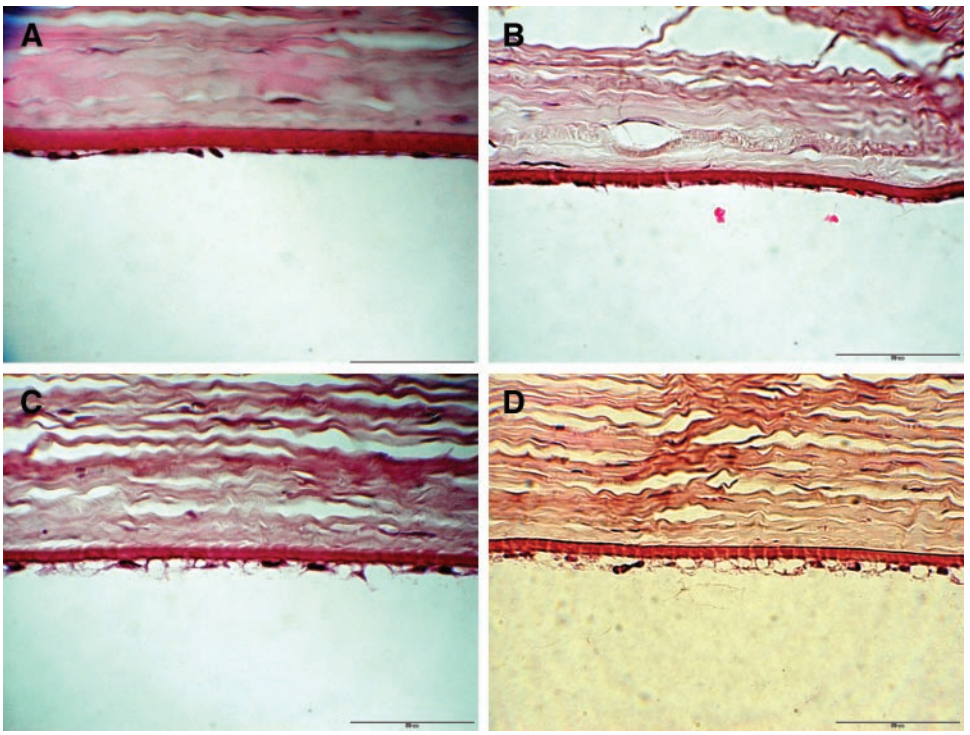

FIGURE 2. Postoperative central corneal sections from the two study groups. *Top*: postoperative corneas from the group treated with solution plus ascorbic acid; *bottom*: corneas from the group treated with BSS. The two latter slides show a marked decrease in endothelial cell density, as well as marked endothelial cell vacuolization. Magnification, ×400.

We have shown that ascorbic acid can reduce the amount of endothelial cell loss after phacoemulsification surgery, and this effect may be due to its free-radical–scavenging properties. Because the two groups were identical in all parameters except for the presence of ascorbic acid in the irrigating solution, the difference in outcome between them strongly suggests that a large part of the endothelial cell loss after phacoemulsification is due to the formation of free radicals (and not solely to thermal and mechanical factors, as previously held). We have also demonstrated that that ascorbic acid sharply reduces the amount of intracellular vacuoles in endothelial cells after phacoemulsification. Because free radicals are known to cause cellular damage by damaging plasma membranes,[5,8,9,12,14] we believe that the presence of these vacuoles indicates the rupture of intracellular organelle membranes.

Further study is needed to establish the concentration of ascorbic acid required. There may be a concentration that provides more endothelial cell protection than we obtained in the current study. The natural concentration of ascorbic acid in human vitreous is approximately 0.001 M,[33,34,37] and we therefore used this concentration for this experiment, although further experiments may reveal a more optimal concentration. It is also possible that combinations of several scavenger chemicals (such as glutathione for example) with ascorbic acid would yield an even greater effect. We believe that continuing studies are warranted to find the optimal protective solution to be used in phacoemulsification cataract surgery.

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