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ARTICLE

Effectiveness of liposomal ozonized oil in reducing ocular microbial flora in patients undergoing cataract surgery

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Purpose: To evaluate the antimicrobial effectiveness of a liposomal ozonized oil solution used as a home therapy in patients undergoing cataract surgery. Antimicrobial efficacy was evaluated as the reduction in the bacterial load of the most common bacteria isolated from cases with endophthalmitis.

Setting: Twenty Italian experimental centers of the Effectiveness of Liposomal Ozonized oil on Ocular Microbial flora before cataract surgery study group.

Design: Interventional, nonrandomized, paired-eye designed, phase 4 clinical study.

Methods: A total of 174 patients undergoing cataract surgery were divided into 2 groups: the study group (174 eyes) underwent surgery and received an isotonic ophthalmic solution of 0.5% ozonized oil in liposomes plus hypromellose treatment (2 drops 4 times/d), and the control group (174 contralateral eyes) was treated with saline solution. The treatment lasted for 3 days. Subconjunctival swabs were taken from both eyes of each patient at T0 (the day before starting the treatment and 4 days preoperatively) and at T4 (after 3 days of treatment and 10 min preoperatively) and sent to the laboratory within 24 hours of collection for microbiological analysis.

Results: 30% of swabs taken at T0 was sterile. Contaminated swabs had a high prevalence of coagulase-negative staphylococci, including *Staphylococcus epidermidis*, and more than 60 different bacterial species were isolated. A significant reduction in microbial load was observed after treatment (>90% of the samples). The microbial load in the control group remained unchanged.

Conclusions: Liposomal ozonized oil reduced the microbial burden after topical administration in a large study population.

J Cataract Refract Surg 2021; ■:1–9

The marked increase in antimicrobial resistance among common bacterial pathogens has become an emergency in clinical practice. In fact, the World Health Organization has classified antibiotic resistance as one of the 3 most important public health threats of the 21st century.[1] Presurgical prophylaxis is crucial to reduce periocular skin and ocular conjunctiva bacterial load before surgical procedures.[2,3] Despite surgical site disinfection and antisepsis of the eyelids and conjunctiva prior to surgery, the incidence of endophthalmitis has increased over the past few decades.[4] Povidone–iodine (PVP–I 5%) solution is currently used in ophthalmic surgery for preoperative prophylaxis because of its wide antimicrobial effect. Topical application of eyedrops containing PVP–I 0.6% reduces the bacterial load on the conjunctiva and enhances the disinfectant activity of PVP–I 5%.[5] Therefore, the discovery of new molecules that are well-tolerated by biological tissues and capable of performing effective antibacterial actions without antibiotic resistance may represent an effective strategy for ophthalmologists.

Ozone is the most powerful oxidizing agent found in nature, with documented antiseptic and anti-inflammatory properties.[6] Ozone gas is a molecule consisting of 3 atoms of oxygen in a dynamically unstable structure because of the presence of mesomeric states. Despite this instability, ozone gas can be stabilized as liposomal ozone dispersion for topical use.[7] Ozonated oils are highly irritant for corneal tissue; thus, a specific formulation has been recently developed for ophthalmic use, made up of ozonized oil 0.5% in liposomes plus

Submitted: February 10, 2021 | Final revision submitted: March 31, 2021 | Accepted: April 2, 2021

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Presented as a poster at the 38th Congress of the ESCRS, Amsterdam, the Netherlands, October 2-4, 2020.

ELOOM: Effectiveness of Liposome Ozonized-oil on Ocular Microbial flora before cataract surgery study investigators are listed in Acknowledgments.

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0886-3350/$ - see frontmatter
https://doi.org/10.1097/j.jcrs.0000000000000672

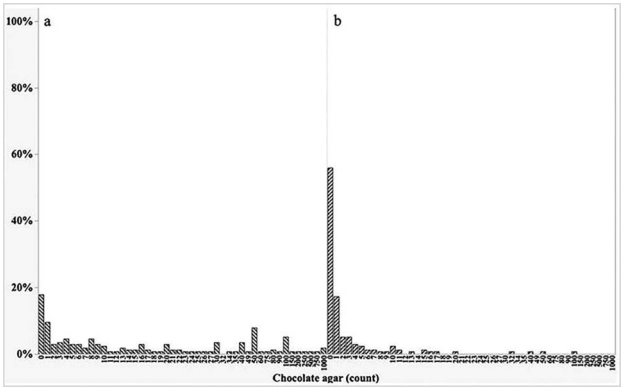

Figure 1. Distribution of percentages of bacterial counts in chocolate agar medium in the control group (*a*) and in the ozonized oil 0.5% in liposomes plus hypromellose group (*b*) at 3 days after treatment (T4).

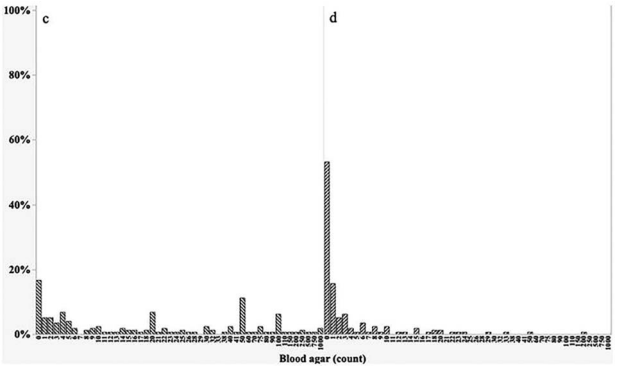

Figure 2. Distribution of percentages of bacterial counts in blood agar medium in the control group (*c*) and in the ozonized oil 0.5% in liposomes plus hypromellose group (*d*) at 3 days after treatment (T4).

hypromellose (Ozodrop, FB VISION S.p.A.).[8] Ozone promotes the release of free oxygen radicals by inducing the synthesis of hydrogen peroxide and lipoperoxide responsible for bacterial lysis and death.[9] In addition, it also helps in the negative regulation of mitochondrial activity in bacteria, disturbance of viral lithic enzymes, and promotion of wound healing because of its ability to release oxygen, platelet-derived growth factor, and transforming growth factor β.[10] Furthermore, its positive action on wound healing minimizes the formation of keloids and, particularly, the risk for corneal haze. Preliminary results have demonstrated that ozone is a safe and effective antiseptic agent in both in vitro and in vivo studies in both animals and humans.[7,11,12] The aim of this study was to evaluate the antimicrobial effectiveness of a new ophthalmic solution of liposomal ozonated sunflower oil plus hypromellose in patients undergoing cataract surgery.

METHODS

Study Design

This was a prospective, interventional, multicentric, nonrandomized, paired-eye designed, phase 4 clinical study named Effectiveness of Liposomal Ozonized oil on Ocular Microbial flora before cataract surgery.

Study Population and Antisepsis Protocol

The study population consisted of patients who underwent cataract surgery and were aged 18 years and older. The eligibility of consecutive patients selected for cataract surgery at 19 academic and nonacademic centers in Italy was evaluated. The names of the participants and the institutions involved are listed in the acknowledgment section. Exclusion criteria included the use of topical or systemic antibiotics, topical antiseptics, current treatment with topical therapies that could not be discontinued for the duration of the study, ocular or systemic inflammatory conditions, infectious processes, or hypersensitivity to the constituents of the study product. Informed consent was obtained from all participants before any study-related procedure was performed, and the nature and purpose of the investigation were fully explained. The study was conducted in accordance with the tenets of the Declaration of Helsinki and the Guidelines for Good Clinical Practice. Ethical approval was obtained in August 2018 from the Scientific Technical Committee of the Department of Medicine and Health Sciences (Vincenzo Tiberio) of the University of Molise, Campobasso, Italy, and in August 2020 from the Ethical Committee of the Sapienza University of Rome, Italy.

Patients were divided into 2 groups. The study group comprised participants undergoing cataract surgery treated with a home therapy of 2 drops of isotonic ophthalmic solution composed of ozonized 0.5% sunflower oil in liposomes plus hypromellose (Ozodrop) 4 times a day (at 8, 12, 4, and 8 PM). The control group (contralateral eyes) was treated with saline solution. The treatment lasted for 3 days. Subconjunctival swabs (eSwab, COPAN Diagnostics) were taken from both eyes of each patient at T0 (the day before starting the treatment and 4 days preoperatively) and T4 (3 days after treatment and 10 minutes preoperatively) and sent to the laboratory within 24 hours for microbiological analysis. Adverse events were recorded during the study period. Ozonized oil in liposomes plus hypromellose is a sterile class 2b medical drug with a CE mark issued by Eurofins (CE 0477), which has been available in the market since September 2017.

Ocular Adverse Events

Ocular adverse events and complications associated with eye drop administration were also recorded. Conjunctival hyperemia was graded into 5 levels: 0, normal conjunctival vessels without engorgement (none); 1, trace flush, reddish pink, minimally dilated blood vessels, and normal underlying sclera easily visible (trace); 2, mild flush, reddish pink, mildly increased density of dilated deep blood vessels, and pink appearance of the sclera (mild); 3, bright red color, significantly tortuous and engorged deep blood vessels, and minimal white scleral tissue visible (moderate); 4, deep bright diffuse redness, a dense network of engorged vessels, and no normal white scleral tissue visible (severe). Subject satisfaction was also evaluated using a 10-point visual analog scale (0 = very comfortable; 1-3 = mild discomfort; 4-6 = moderate discomfort; and 7-10 = severe discomfort) at the postbaseline visit.

Bacterial Cultures

From June 2019 to December 2020, 174 patients were received at the C.S. Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy. Conjunctival swabs of both eyes were taken from each patient (carefully avoiding contact of the swab with the eyelashes) using an eSwab. An eSwab consists of a sterile package containing 2 components: a prelabeled polypropylene screw-cap tube with a conical or round bottom filled with 1 mL of liquid transport medium and a specimen collection swab, which has a tip flocked with soft nylon fiber. The transport medium was a maintenance medium composed of inorganic phosphate buffer, calcium and magnesium salts, and sodium chloride in a reduced environment because of the presence of sodium thioglycolate and can sustain the viability of a plurality of organisms. The total number of samples received by the laboratory was 460 (230 conjunctival

swabs at 2 different times: T0 and T4). Immediately on receipt at the laboratory (refrigerated transport), the samples were placed on a stirrer to homogenize the transport medium, of which 300 μL were subsequently plated on chocolate agar and 300 μL on BD Centers for Disease Control (CDC) anaerobe agar + 5% sheep blood (SB). Chocolate agar is a nonselective medium for the isolation of fastidious organisms from clinical specimens, containing proteose peptone, digested liver, and yeast extract as sources of nitrogen and vitamins. Osmotic balance was maintained using sodium chloride. Heat-denatured horse blood provides, in addition to various nutrients, factor X (heme) and factor V (nicotinamide adenine dinucleotide, NAD). BD CDC anaerobe agar + 5% SB is a nonselective medium for the isolation and culture of fastidious obligate anaerobes from clinical specimens. CDC anaerobic agar with 5% SB has been formulated as an enriched, nonselective medium for the isolation and culture of a wide range of obligate anaerobic bacteria. The medium contained trypticase soy agar enriched with agar as a nourishing base. Osmotic balance was maintained with sodium chloride. SB, hemin, cystine, and vitamin K1 were provided as growth factors needed for obligate anaerobes. A volume of 300 μL was carefully evaluated to allow the operator to obtain an accountable number of bacterial colonies; values less than and more than 300 μL do not allow for a correct estimate of the bacterial load present in the conjunctiva. The plates were incubated for 48 hours at 37°C in anaerobiosis (CDC anaerobe + 5% SB) or at 37°C with 5% CO_2 (chocolate agar). Colonies were counted macroscopically, and subsequently, colony-forming microorganisms were identified using matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. MALDI-TOF is a powerful analytical mass spectrometry technique that measures the mass of molecules from a sample that has been embedded in a matrix by using a laser to ablate and desorb molecules with minimal fragmentation.[13] To perform the MALDI-TOF analysis, a colony was picked from a culture plate to a spot on a MALDI-TOF target plate; multiple strains can be tested simultaneously on the same slide. Subsequently, 1 μL of matrix was added (substance rich in protons to favor the ionization of the sample and with great absorption to absorb the laser radiation with great efficiency), and the plate was placed in the ionization chamber of the mass spectrometer. The sample molecules were ionized in the resultant hot plume of ablated gases and funneled into a TOF mass spectrometer that recorded the ion mass-to-charge (m/z) ratio. This was achieved by measuring the time taken by the ions to traverse a known length under acceleration by an electric field of known strength. The resultant mass spectrum was produced from the pattern (ie, position and relative intensity) of the detected m/z peaks, generating a distinct profile for a particular sample. The uniqueness of mass spectra can be leveraged for identification purposes when a comparison reference spectrum is available. All results obtained were compared with a database of mass spectra using software, resulting in identification of the organism.

Sample Size

The study was configured as a paired-eye design; in each patient, 1 eye was treated with ozonized oil in liposomes and hypromellose, whereas the contralateral eye was used as the control. The study evaluated the time difference (the day before starting the treatment vs after 3 days of treatment) between treatment with ozonized oil in liposomes and hypromellose and control. Since the SD of the treatment–control differences between the preoperative and postoperative differences (generally not known) was not known, a pilot study was conducted to obtain these unknown data.[14] To make the data less variable as the bacterial count was positively skewed, a logarithmic transformation was applied. The SD of the differences was estimated as ±0.69. A positive correlation exists between the 2 eyes, which was also confirmed in the pilot study.[15,16] Assuming a mean difference (clinically plausible) between treatment and no treatment (d = 0.16), a sample size of $n_{pairs} \cong 145$ was needed, with α = 0.05 and a power of 80%, as follows:

$$n_{pairs} = \frac{Z_{1-\frac{\alpha}{2}} - Z_{1-\beta} \times \mathrm{SD}^2_{\mathrm{diff}}}{d^2}.$$

Considering a dropout rate of 20%, the sample size was calculated to be at least 174 pairs of eyes.

Statistical Analysis

Continuous variables are presented as mean ± SD and/or median (95% IC, min to max). Categorical variables are presented as absolute frequencies and percentages. Figure 1, a and b and Figure 2, c and d illustrate the distributions of colony counts in the 2 groups (control [a, c] after 3 days and ozonized oil 0.5% in liposomes plus hypromellose [b, d] after 3 days of treatment [T4]). Both groups indicate highly positive skewness, with most values being low, relatively few high values, and a large proportion of zeros. In such cases, a zero-inflated model was applied to analyze the data to avoid model misspecification due to the presence of excess zeros, which may result in biased or inconsistent estimators. In this study, a zero-inflated negative binomial (ZINB) model was used with repeated measurements from the same subject. The ZINB model is particularly appropriate for variables with 2 different processes: one that influences the occurrence and the other that influences the frequency of occurrence. The distribution of the outcome variable was approximated by mixing the 2 models and 2 distributions. The first model is based on logistic regression and predicts nonoccurrence.[17] A combination of a generalized linear model and a nonlinear mixed model was used to estimate the correlation of colony count in chocolate and blood agar within a subject.[18–20] This combination was necessary as there is no single procedure in which the ZINB distribution and the correlation between the eyes were considered simultaneously. Generalized linear model was applied to estimate parameters that were consequently substituted in the nonlinear mixed model, where the ZINB distribution was implemented along with the correlation between the eyes.

The second model examined the frequency of an event occurring using negative binomial regression. Negative binomial regression is an extension of Poisson regression. The negative binomial regression possesses greater flexibility than Poisson regression in modeling the relationship between conditional variance and conditional mean.[17] The ZINB model produces 2 sets of coefficients: 1 predicting whether the event occurred (logistic part) and 1 predicting the frequency of occurrence of the event (negative binomial). As the ZINB model is a mixed model, the predictors for the 2 parts of the model may be different.[17] Colony count at baseline was considered as a covariate within this study because, at this temporal point, the effect of treatment was not observable.[21,22] To confirm the results, another method was applied based on the proportional odds ratio. The count variable (chocolate and blood agar on the day before starting the treatment and after 3 days of treatment) was discretized into 3 classes (0-10, 11-100, and 101-1000). Then, a multinomial logistic regression was applied considering the command REPEATED to consider correlated eyes. This regression considered a response variable with more than 2 categories on an ordinal scale. The cumulative logit proportional odds model fits the data in this study.[23,24] The scope was to evaluate the odds ratio of the treatment (ozonized oil 0.5% in liposomes plus hypromellose vs control) relative to the response variable and count variable at baseline as a covariate. Comparisons of chocolate and blood agar between the day before starting the treatment vs the day before starting the treatment and after 3 days of treatment vs after 3 days of treatment were evaluated using the Wilcoxon test. Poisson regression was used to evaluate the differences between the ratios of the species in accordance with the treatment and to count the classes of chocolate and blood agar after 3 days of treatment (T4).

Table 1. Bacterial Species Isolated in this Study.

Gram-positive (52 species)		Gram-negative (12 species)
Coagulase-negative staphylococci: *Staphylococcus epidermidis*, *Staphylococcus saccharolyticus*, *Staphylococcus hominis*, *Staphylococcus pasteuri*, *Staphylococcus capitis*, *Staphylococcus caprae*, *Staphylococcus warneri*, *Staphylococcus lugdunensis*, *Staphylococcus pettenkoferi*, *Staphylococcus saprophyticus*, *Staphylococcus schleiferi*, *Staphylococcus cohnii*, and *Staphylococcus haemolyticus*	*Micrococcus* spp.	*Pseudomonas aeruginosa*
Staphylococcus aureus	*Propionibacterium acnes*	*Haemophilus influenzae*
Streptococcus spp., *Streptococcus salivarius*, *Streptococcus faecalis*, *Streptococcus sanguinis*, *Streptococcus parasanguinis*, *Streptococcus mitis*, *Streptococcus pneumoniae*, *Streptococcus intermedius*, *Streptococcus oralis*, and *Streptococcus gordonii*	*Finegoldia magna*	*Neisseria macacae* and *Neisseria subflava*
Granulicatella spp.	*Microbacterium hydrocarbonoxydans*	*Veillonella* spp., *Veillonella atypica*, and *Veillonella dispar*
Kytococcus schroeteri	*Corynebacterium* spp., *Corynebacterium macginleyi*, *Corynebacterium bovis*, and *Corynebacterium propinquum*	*Proteus mirabilis*
Micrococcus spp. and *Micrococcus luteus*	*Cutibacterium acnes* and *Cutibacterium avidum*	*Escherichia coli*
Kocuria spp., *Kocuria rhizophila*, *Kocuria palustris*, and *Kocuria kristinae*	*Bacillus megaterium*, *Bacillus licheniformis*, *Bacillus cereus*, and *Bacillus subtilis*	*Pasteurella canis*
Rothia spp., *Rothia mucilaginosa*, *Rothia dentocariosa*, and *Rothia aeria*	*Actinomyces odontolyticus*	*Klebsiella oxytoca*
Dermabacter hominis		*Moraxella nonliquefaciens*

Statistical analysis was performed using SAS v. 9.4 and JMP Pro v.15 (SAS Institute). A P value of less than 0.05 was considered statistically significant.

RESULTS

Swabs taken at T0 were sterile in 30% of cases. The contaminated swabs presented with a high prevalence of coagulase-negative staphylococci (CoNS; including *Staphylococcus epidermidis*), in accordance with the ESCRS data.[25] CoNS were isolated in 66.8% of the swabs, *Staphylococcus aureus* in 13.9%, and other species including gram-positive bacteria such as *Streptococcus mitis* and *Micrococcus* spp. and gram-negative bacteria such as *Pseudomonas aeruginosa* and *Proteus mirabilis* were identified at lower rates. Table 1 lists the bacterial species isolated in this study. More than 90% of the samples presented a significant reduction (>90%) in microbial load after liposomal ozonized oil topical treatment. The microbial load in the control group remained unchanged. The ocular tolerability of the solution was optimal because of its liposomal nature. Figure 3 illustrates the results of multinomial logistic regression with chocolate and blood agar classes at 3 days (T4) as response variables. In both classes, the treatment with ozonized oil 0.5% in liposomes plus hypromellose induced a statistically significant difference ($P < .0001$), with an odds ratio of 28.24 (95% CI 11.40 to 69.96) and 23.17 (95% CI 10.51 to 51.05), respectively.

Tables 2 and 3 report the estimated parameters of ZINB regression for chocolate and blood agar at the end of the study, showing that the estimate of treatment variables (β_2) was significant ($P < .0001$ for both). Chocolate and blood agar were also associated with response variables ($P = .0001$ and $P = .0002$, respectively). In the logistic part of Tables 2 and 3, the positive estimate values (g_2) were significant ($P = .026$ and $P = .0021$, respectively). In these models, the counts at baseline were associated with both chocolate and blood agar ($P = .0001$ and $P = .0002$, respectively). The coefficients for the count part of the model can be interpreted by exponentiating the regression coefficients by placing the predicted values for the outcome on its original scale. The coefficients for the logistic regression were on the logit scale, thus exponentiating the transformed values to

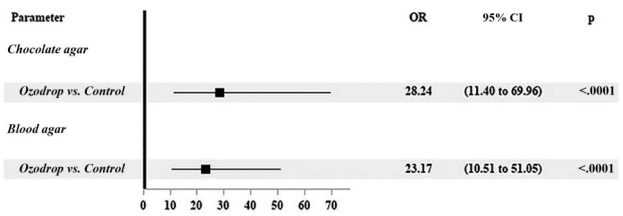

Figure 3. Odds ratios (ORs) in multinomial logistic regression according to classes of chocolate and blood agar after 3 days of treatment (T4).

Table 2. The β and γ Estimates for Chocolate Agar Count Blood.

Parameter	Mean ± SE	95% CI	Exp(mean)	%	*P* value	Gradient
Count part						
β0	2.94 ± 0.16	2.63, 3.25	23.81	2280.75	**<.0001**	−1.95E-06
β1	0.003 ± 0.0009	0.002, 0.005	1.00	0.30	**.0001**	1.90E-06
β2	−2.51 ± 0.20	−2.91, −2.10	0.08	−91.87	**<.0001**	−1.52E-06
σu^2	1.10 ± 0.12	0.85, 1.35			**<.0001**	2.31E-06
Logistic part						
γ0	−1.19 ± 0.48	−2.14, −0.24			**.014**	4.23E-06
γ1	−0.26 ± 0.13	−0.51, −0.006			**.045**	8.86E-06
γ2	1.37 ± 0.61	0.16, 2.57			**.026**	2.65E-06
σv^2	0.82 ± 0.99	−1.14, 2.77			.41	1.35E-06
K	1.17 ± 0.20	0.77, 1.56			**<.0001**	2.91E-06

β values represent the coefficients for the count part whereas γ values are coefficients for the zero part
Count part: log(μi) = β0 + β1 × chocolate agar at baseline + β2 × treatment + ui
Logistic part: logit(probi) = γ0 +γ1*chocolate agar at baseline + γ2*treatment + vi

odds ratios. The logistic part predicted the nonoccurrence of the outcome; these coefficients must be interpreted carefully as the results were modeled on the logit scale.

The presence of excess zeros indicates a high probability of overdispersion. Analysis of the data showed that the variance was notably higher than the mean (chocolate agar at the end of the study: ozonized oil in liposomes plus hypromellose group, mean = 2.98 and variance = 90.93, control group, mean = 45.62 and variance = 20 827.01; blood agar at the end of the study: ozonized oil in liposomes plus hypromellose group, mean = 4.22 and variance = 259.76, control group, mean = 51.01 and variance = 2980.90); this shows overdispersion as a model with Poisson distribution was not adapted. The odds ratios of 28.24 and 23.17 indicated that ozonized oil in liposomes plus hypromellose scored significantly better than the control. In Tables 2 and 3, the values of $\beta_2 = -2.51 \pm 0.20$ (chocolate agar) and $\beta_2 = -2.51 \pm 0.19$ (blood agar) were significant in both regressions, indicating that ozonized oil in liposomes plus hypromellose reduced bacterial counts relative to the control. The model fitted the data appropriately as the k value (dispersion) was approximately one, the variances (σu^2 and σv^2) were low, and all gradients were lower than 10 to 3. In both chocolate and blood agar, treatment with ozonized oil in liposomes plus hypromellose reduced the bacterial count by nearly 92% after 3 days, as summarized in Tables 2 and 3. The positive estimate g_2 in Tables 2 and 3 (1.37 and 1.77 for chocolate and blood agar, respectively) indicated that there was a probability for the subjects treated with ozonized oil in liposomes plus hypromellose to be in the zero-species class. Figures 1 and 2 show a greater reduction in colony counts in the eyes treated with ozonized oil in liposomes plus hypromellose than in the controls. This trend was confirmed by the methodologies applied to the count data in Tables 2 and 3.

Only 3 (1.72%) of the 174 patients reported ocular adverse events in the treated eye; minimal conjunctival hyperemia was detected. As summarized in Table 4, no other signs of conjunctival involvement (discharge and papillae) were recorded. Comfort analysis showed that 167 patients (95.98%) did not experience any ocular discomfort, whereas mild discomfort occurred in 6 patients (3.45%). No corneal abnormalities or other significant side effects were observed. None of the 174 patients showed signs of endophthalmitis after the cataract surgery procedure.

Table 3. The β and γ Estimates for Blood Agar Count Blood.

Parameter	Mean ± SE	95% CI	Exp(mean)	%	*P* value	Gradient
Count part						
β0	3.17 ± 0.15	2.87, 3.47	23.57	2257.06	**<.0001**	9.54E-07
β1	0.002 ± 0.0007	0.001, 0.004	1.00	0.30	**.0002**	7.20E-05
β2	−2.51 ± 0.19	−2.88, −2.15	0.08	−91.87	**<.0001**	1.24E-06
σu^2	1.11 ± 0.13	0.86, 1.35			**<.0001**	−9.14E-08
Logistic part						
γ_0	−1.47 ± 0.58	−2.61, −0.33			**.012**	3.67E-07
γ_1	−0.32 ± 0.13	−0.58, −0.06			**.017**	3.63E-06
γ_2	1.77 ± 0.76	0.27, 3.28			**.021**	6.99E-08
σv^2	1.91 ± 0.88	0.17, 3.65			**.032**	8.57E-07
K	1.12 ± 0.19	0.84, 1.59			**<.0001**	−9.60E-08

β values represent the coefficients for the count part whereas γ values are coefficients for the zero part
Count part: log(μi) = β0 + β1 × blood agar at baseline + β2 × treatment + ui
Logistic part: logit(probi) = γ0 +γ1 × blood agar at baseline + γ2 × treatment + vi

Table 4. (A) Report on Ocular Adverse Events (OAEs) and (B) Report on the Analysis of Visual Analog Scale (VAS) in the Ozonized Oil 0.5% in Liposomes Plus Hypromellose Group.

	Grade					
(A) OAE	None	Trace	Mild	Moderate	Severe	*P* value
Conjunctival hyperemia, n (%)	171 (98.28)	3 (1.72)	0 (0.00)	0 (0.00)	0 (0.00)	**<.0001**
Conjunctival discharge, n (%)	—	—	—	—	—	
Conjunctival papillae, n (%)	—	—	—	—	—	
Corneal changes, n (%)	—	—	—	—	—	
Keratitis, n (%)	—	—	—	—	—	
Eye pain, n (%)	—	—	—	—	—	
(B) VAS	**Very comfortable**	**Mild discomfort**	**Moderate discomfort**	**Severe discomfort**	***P* value**	
Comfort, n (%)	167 (95.98)	6 (3.45)	1 (0.57)	0 (0.00)	**<.0001**	

Post hoc analysis: comfort to very comfortable vs mild discomfort: $P < .0001$; very comfortable vs moderate discomfort: $P < .0001$.

DISCUSSION

The periocular zone and conjunctiva have significant microbial loads, which constitute the ocular microbiota and play an important role in maintaining the equilibrium of the ocular surface.[26] In particular, *S aureus* and CoNS are the most frequently isolated species.[27] To minimize the risk for infection, it is crucial to decrease the conjunctival bacterial load. According to the ESCRS guidelines for the prevention and treatment of endophthalmitis after cataract surgery, a mandatory step to reduce bacterial load in the wound area is to apply PVP–I 5% to 10% onto the cornea, conjunctival sac, and periocular skin, at least 3 minutes prior to surgery. If PVP–I is contraindicated (true allergy is rare, and hyperthyroidism represents a relative contraindication to a 1-time administration), aqueous chlorhexidine 0.05% may be used.[25] PVP–I is an effective antiseptic used in general and ophthalmic surgery for infection prophylaxis.[28]

Preclinical data indicate that PVP–I has a broad antimicrobial activity against gram-positive and gram-negative bacterial isolates and adenoviruses.[29,30] Recently, Ta et al. reported a randomized, double-masked, multicenter, phase 3 clinical trial to evaluate the efficacy and safety of a topical ophthalmic suspension combination of PVP–I 0.6% and dexamethasone (DEX) 0.1% for acute bacterial conjunctivitis.[31] Subjects included in the study were randomized into 3 groups to receive PVP–I 0.6% /DEX 0.1%, PVP–I 0.6% alone, or placebo. Benzalkonium chloride (0.01%) was added to the placebo as a preservative but was not included in the PVP–I/DEX or PVP–I treatments as PVP–I acts as a preservative. The primary end point was clinical resolution in the study eye, and the key secondary efficacy end point was bacterial eradication, evaluated after 5 days of treatment. The study showed no statistically significant differences between the treatment groups for both the primary and secondary end points. The authors hypothesized that the outcome of the study was determined by an insufficient dose of PVP–I, and they suggested that the free iodine concentration peak in this dose range and the cumulative antimicrobial effect may have been short-lived because the available iodine is quickly used up in the reaction with bacteria.[31] This could explain the lack of efficacy of PVP–I/DEX in this study. Furthermore, as benzalkonium chloride is an antimicrobial agent and a cationic surfactant, it may be efficient in the treatment of bacterial conjunctivitis, thereby contributing to the lack of difference in efficacy between the placebo and treatment groups.[31] Reibaldi et al. verified the efficacy of preservative-free PVP–I 0.6% eyedrops in reducing the conjunctival bacterial load in patients undergoing intravitreal antivascular endothelial growth factor injection.[5] The study was conducted on 508 patients (254 treated and 253 controls), and bacterial growth from conjunctival swab cultures was significantly lower in the PVP–I-treated group than at baseline and the control group.

The search for additional topical treatments with a broad-spectrum antimicrobial activity, acceptable tolerability, and low potential for promoting resistance has led to ozonized oils, experiencing an increase in scientific interest and clinical applications. Ozone in both gaseous and aqueous phases has been demonstrated to be a powerful and reliable antimicrobial agent against bacteria. It is capable of killing all known types of gram-positive and gram-negative bacteria, including extremely resistant bacteria such as *Escherichia coli* and multiresistant *S aureus*.[32–35] This antimicrobial effect is related to ozonolysis or the disruption of the integrity of the bacterial cell envelope through oxidation of the phospholipids and lipoproteins, in addition to damaging the bacterial cytoplasmic membrane.[32] This action is nonspecific and selective to microbial cells and could also play an important role in cases of resistant bacteria.[35]

Ozone has been demonstrated to inhibit cell growth in fungi at certain stages and to induce damage to viral capsids by interrupting their reproductive cycle and disrupting virus-to-cell contact by peroxidation.[32] Celenza et al. studied the antifungal activity of ozonized oil 0.5% in liposomes plus hypromellose against 4 clinical *Candida* spp.: *Candida albicans*, *Candida glabrata*, *Candida krusei*, and *Candida orthopsilosis*. All *Candida* isolates were susceptible to ozonized oil 0.5% in liposomes plus hypromellose, and after a 1-hour exposure at the minimum inhibitory

concentration, approximately 30% of cells were killed, reaching approximately 70% at the highest ozonized oil 0.5% in liposomes plus hypromellose. *Candida albicans* showed cell membrane depolarization, increased levels of lipids peroxidation, depolarized $\Delta\psi m$, and increased generation of reactive oxygen species. The antifungal activity of the ozonized sunflower oil eyedrops causes alteration in the cell membrane structure. This is probably due to peroxidation of unsaturated membrane lipids, which leads to deformation of the structure and functionality of the plasma membrane.[36]

Liposome-vesiculated ozonated oil has shown antimicrobial efficacy against bacterial strains of *S aureus* and *Pseudomonas aeruginosa*. The bactericidal action against *P aeruginosa* was lower than that against *S aureus*. Bactericidal activity was obtained at a bacterial concentration of 150 colony-forming units (CFU)/mL for *S aureus* but at a concentration of 15 CFU/mL for *P aeruginosa*. The effect was dose dependent regarding the volumes of the liposome-vehiculated ozonated oil added to 100 μL of bacterial suspension (400 μL, 200 μL, 100 μL, and 50 μL, respectively); the higher the dose, the higher the bactericide action. Moreover, a study performed in vitro on a keratinocyte line showed that ozonated oil in liposomes does not show any cell toxicity.[37] The same formulation has also been used to promote wound healing and to treat some inflammatory and infectious pathologies of the anterior segment in both humans and animals, including vernal conjunctivitis, granulomatous conjunctivitis, and persistent dystrophic corneal ulcer.[7,12] These preliminary in vivo studies demonstrated that ozone-based eyedrops have anti-inflammatory and bactericidal activity and promote tissue repair.

Because of its great oxidative power, ozonized oil is used topically for the treatment of wounds and anaerobic and viral infections.[38] In addition to its bactericidal activity, the effectiveness of ozonated oil could also be attributed to its ability to dissolve biofilms.[39] It is very likely that both mechanisms of action (ie, bactericidal activity and biofilm dissolution) contribute to defining the elevated safety and efficacy profile of ozonated oil.

The formulation of liposomal ozone dispersion did not show any corneal damage, inflammation, or other signs of cell toxicity and has been demonstrated to reduce the bacterial load on the ocular surface in dogs.[40] This suggests the potential use of liposomal ozone dispersion as a preoperative cleaning agent, because of the lack of adverse events and the high satisfaction rate recorded (Table 4).

In conclusion, our results demonstrated that topical administration of liposomal ozonized oil treatment in a large study population induced a significant reduction in conjunctival microbial load, reducing the load of pathogens that could give rise to a perioperative infection. The antiseptic activity, together with the lack of toxicity or allergenic power, suggests that liposomal ozonized oil could be considered a safe and effective adjuvant in home prophylaxis, thus improving preoperative prophylaxis procedures in cataract surgery.

Acknowledgments

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WHAT WAS KNOWN

- The World Health Organization has classified antibiotic resistance as one of the 3 most important public health threats in the 21st century. Presurgical prophylaxis is crucial to reduce periocular skin and ocular conjunctiva bacterial load before surgical procedures. Ozone is the most powerful oxidizing agent found in nature, with documented antiseptic and anti-inflammatory properties.

WHAT THIS PAPER ADDS

- Topical application of liposomal ozonized oil can reduce the microbial burden before cataract surgery, with no toxic or allergenic activities. Therefore, it could be considered a safe and effective adjuvant for home prophylaxis in cataract surgery.

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Disclosures: *None reported.*

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000 Effectiveness of liposomal ozonized oil in reducing ocular microbial flora in patients undergoing cataract surgery

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Topical liposome ozonized oil application, reducing microbial burden before cataract surgery without any toxicity or allergenic activity, could be considered a safe and effective adjuvant in home prophylaxis for cataract surgery.