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Corneal endothelial cells' protection against thermal injury: Influence of ophthalmic viscoelastic substances in experimental study on rabbits

P. JUROWSKI[1], R. GOŚ[1], G. OWCZAREK[2], G.Z GRALEWICZ[2]

[1]Department of Ophthalmology and Visual Rehabilitation, Medical University of Lodz
[2]Central Institute for Labour Protection, National Research Institute, Warsaw - Poland

PURPOSE. *To quantify the maximal level of temperature and the time the maximal temperature is achieved and correlate the temperature parameters to the mean rate of endothelial cell loss after standardized ultrasound delivery assisted with four viscoelastic substances or different temperature of balanced salt solution (BSS).*

METHODS. *Thirty rabbits (60 eyes) were divided into six groups in which different viscoelastic substances or different temperature of BSS were used: Group 1, Viscoat; 2, Provisc; 3, soft shell technique; 4, Celoftal; 5, BSS 22 °C; and 6, BSS 4 °C. The same parameters of ultrasound energy were delivered by standard phaco tip introduced into pupillary plane. Thermocamera was employed for measurements of temperature parameters. Endothelium cell count was measured before surgery and 1 month postoperatively.*

RESULTS. *Maximal level of temperature was measured as follows: Group 5, 27.85 ± 0.52 °C; Group 2, 27.75 ± 0.54 °C; Group 3, 27.74 ± 0.46 °C; Group 4, 27.25 ± 0.60 °C; Group 6, 26.81 ± 0.34 °C; Group 1, 26.52 ± 0.48 °C ($p<0.05$). The time the maximal temperature is achieved was statistically shorter in Groups 5 and 6: 4 seconds, 5 seconds, respectively, $p<0.0001$, as compared with Group 2 (30 seconds), Group 3 (40 seconds), Group 1 (45 seconds), and Group 4 (50 seconds). The mean rate of endothelial cell loss was calculated as follows: Group 1, 4.35%±2.55%; Group 2, 8.43%±5.2%; Group 3, 6.25%±4.20%; Group 4, 6.53%±4.65%; Group 5, 14.3%±3.85%; and Group 6, 8.78%±4.45%.*

CONCLUSIONS. *Viscoelastic substances offer different levels of endothelial cell protection against temperature increase during phacoemulsification. The mean rate of endothelial cell loss correlates with the time the maximal temperature is achieved rather than with the value of maximal level of temperature. This implicates that surgical strategy should consider the choice of the most effective viscoelastic substances, particularly in difficult cases, e.g., hard nucleus, shallow anterior chamber, primary endothelial abnormality. (Eur J Ophthalmol 2005; 15: 674-9)*

KEY WORDS. *Corneal endothelium, Viscoelastic substances, Thermography*

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INTRODUCTION

Protecting endothelial cells from surgical trauma during phacoemulsification is essential in the cataract surgical procedure. Despite advancement in surgical techniques and modern surgical devices, corneal endothelial cell loss followed by corneal decompensation is still one of the most frequent complications.

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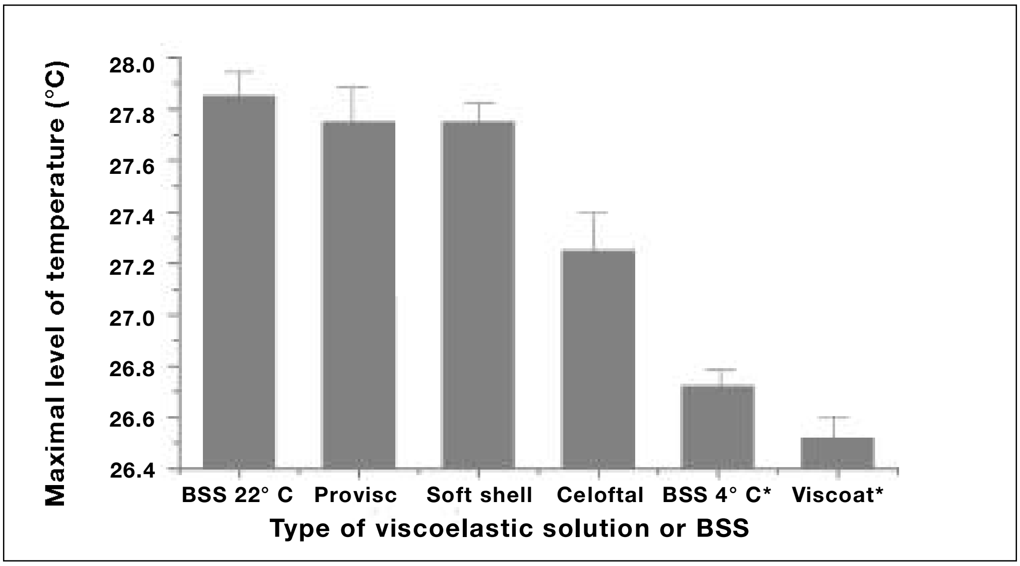

Fig. 1 - *Mean values of maximal level of temperature during ultrasound delivery. *p<0.05 (mean ± SD).*

The benefits of viscoelastic substances use during phacoemulsification include protection of corneal endothelium and structures of anterior chamber against direct mechanical trauma, and several indirect factors, e.g., ultrasound energy (1), free radicals induction (2, 3), and acoustic cavitation (4).

The increase of temperature within anterior chamber during phacoemulsification is a well known and potentially relevant factor of sensitive corneal cell damage (5, 6). In this experimental study the authors compared the efficacy of viscoelastic substances or different temperature of irrigating solutions in protecting corneal endothelial cells against the increase of temperature after delivering standardized ultrasound energy.

METHODS

Thirty New Zealand white rabbits (60 eyes) (2.5 to 3.0 kg) were used in this study. Based on different viscoelastic or irrigating solutions used in the study, the rabbits were randomly divided into six treatment groups, each including 5 rabbits/10 eyes: Group 1, Viscoat (4% chondroitin sulfate and 3% sodium hyaluronate); Group 2, Provisc (1% sodium hyaluronate); Group 3, dispersive-cohesive viscoelastic substances injected sequentially without mixing (soft shell technique); Group 4, Celoftal (1% hydroxypropyl methylcellulose); and in Groups 5 and 6, only balanced salt solution (BSS) was used at 22 °C and 4 °C, respectively.

The study was performed in accordance with the University Ethics Committee regulations (ŁB 160).

Surgical technique

The rabbits were anesthetized with intramuscular injection of a mixture of ketamine hydrochloride (30 mg/kg) and xalazine hydrochloride (10 mg/kg) and topical instillation of propacaine hydrochloride 0.5% (Alcaine) as a local anesthetic agent. Before surgery, pupil dilation was achieved by instillation of cyclopentholate hydrochloride 1% and phenylephrine hydrochloride 10% every 5 minutes for 30 minutes. The last application of any drops was applied 20 minutes before surgery. As the study was to assess the influence of different viscoelastic substances and different temperature of BSS on the rate of corneal endothelial cell loss, both eyes were operated on at the same time. All surgical procedures were performed in the same manner and in the same circumstances (room temperature 22 °C, humidity 50%). The continous curvilinear capsulorhexis with cystotome was conducted by lateral puncture followed by superior clear corneal 2.7 mm incision. Lens nucleus and cortical materials were aspirated by bimanual aspiration-irrigation maneuvres. After the anterior chamber was carefully filled with different viscoelastic substances or irrigating solution alone, ultrasound phaco tip (Series 10 000 Master Cavitron Phacoemulsifier, Alcon Laboratories) was introduced in the iris plane and switched on. The same standardized machine settings were maintained: US power 60%, linear phaco, 90 seconds, maximal vacuum level 50 mmHg. BSS was used as an irrigating solution 25 mL/min at 22 °C, except in Group 6, where it was chilled to 4 °C. To retain the temperature of irrigating solutions at the level of 4 °C, BSS bottles, phaco tip, and tubes were chilled in a refrigerator set to 4 °C 24 hours before surgery. Two bottles of chilled BSS were used: one during nucleus and cortical material removal and the second one just before the ultrasound energy delivery. The temperature of BSS was checked just before and at the conclusion of the surgery by electronic thermometer.

Outcome parameters

Thermocamera ThermaCam Inframetrics 290E was employed to measure the mean temperature within circular area of 10 mm diameter on the surface of the central cornea. The temperature range and standard deviation were also calculated. Maximal level of the temperature (MLT) was measured just before and during surgery. When the ultrasound energy was delivered intraoperatively the temperature readings were captured continuously. Further analysis of the temperature readings allowed for the assessment of the time the maximal temperature is achieved (TMTA).

The temperature resolution of thermography system was 0.01 °C. All thermal images were captured and processed through high speed Peripheral Component Interconnect (PCI) interface. This interface links the thermal camera with a powerful computer, and offers a high performance of 32-bit data transfer. ThermalStudio software was used.

Endothelial cell count was obtained with contact specular microscope (Tomey 1100). More than 100 cells were analyzed using a cell analysis system to assess the mean rate of endothelial cell loss. The measurements were performed before surgery and 1 month postoperatively.

Statistical analysis

A two-way repeated-measures analysis of variance (ANOVA) was used to determine whether there were statistically significant differences among six groups in the mean rate of endothelial cell loss and maximal level of temperature and the time the maximal temperature was achieved. A p value less than 0.05 was considered statistically significant.

RESULTS

There were no differences in mean temperature between eyes preoperatively (22.76 ± 1.48 °C). A significant increase of the MLT was noted when the working phaco tip was switched on in each group as compared with preoperative temperature ($p<0.05$). The highest MLT was observed in Group 5. There were no significant differences in MLT among Groups 2, 3, 4, and 5. Significantly lower MLT was obtained in Groups 1 and 6, respectively, as compared with Group 5 (Fig. 1).

The TMTA was significantly shorter in Groups 5 and 6, where only BSS was used 4 seconds and 5 seconds, respectively ($p<0.0001$), as compared with the groups in which surgery was performed assisted with viscoelastic substances as follows: Provisc, soft shell, Viscoat, Celoftal: 30 seconds, 40 seconds, 45 seconds, 50 seconds, respectively.

Mean rate of endothelial cell loss 1 month after surgery was statistically lower in Viscoat, soft shell technique, Celoftal, groups: 4.35%±2.55%, 6.25%±4.20%, 6.53%±4.65%, respectively, as compared to Provisc

TABLE I - CENTRAL CORNEAL ENDOTHELIAL CELL DENSITY AND LOSS RATE

Parameter	Provisc	Viscoat	Celoftal	Soft shell	BSS 22 °C	BSS 4 °C
Mean corneal endothelial cell density (per mm^2)						
Preop.	2571±135	2627±215	2748±230	2646±147	2555±200	2614±165
1 month postop.	2355±198	2513±96	2569±167	2480±104	2190±94	2385±110
Mean rate of endothelial cell loss 1 month postop. (%)	8.43±5.2	4.35±2.55	6.53±4.65	6.26±4.20	14.3±3.85	8.78±4.45
p value	<0.05				<0.001	<0.001

Significantly lower value of the mean rate of endothelial cell loss was observed for dispersive viscoelastic solutions and soft shell technique as compared with Provisc and both BSS. Values are means ± SD

8.43%±5.2% and cold BSS 8.78%±4.45% ($p<0.05$). Significantly higher values were noted in groups in which normal temperature of BSS was used (14.3%±3.85%) ($p<0.001$) (Tab. I).

DISCUSSION

Increase of temperature is a common finding during phacoemulsification (7) that may contribute to wound burn, postoperative blood-aqueous barrier breakdown (8), and endothelial cell damage (9). There are currently some methods to diminish intraoperative temperature, e.g., reducing phaco power; delivering ultrasound energy in pulse, burst, or hyperpulse modes (10); providing laser phacolysis surgery (11); or using protecting solutions, e.g., viscoelastic substances. While the use of viscoelastic substances is a universally accepted method of phacoemulsification protection, different physicochemical properties of viscoelastic substances may provide different levels of endothelial cells protection against thermal stress. Using an animal model that allows delivery of a standardized amount of ultrasound energy and employing thermocamera for measurements of thermal parameters, we have demonstrated statistically significant quantitative differences in MLT among four groups of different viscoelastic materials or two groups with different temperatures of BSS. Among viscoelastic substances groups, Viscoat exhibited significantly lower level of MLT as compared with Celoftal, soft shell technique, and Provisc. However, the differences among the last three groups were not statistically significant. This difference in MLT may potentially depend on some rheologic properties of currently used viscoelastic substances, e.g., adhesion, retention, and temperature transmission, and may suggest that Viscoat provides the most effective isolatory behavior. The findings of this study may be supported by the previous assessment of McDermott et al that the amount of adherent viscoelastic substances is highly dependent on viscoelastic cohesive/dispersive type (12), and Glasser et al regarding greater intraocular retentive tendency of Viscoat than cohesive viscoelastic substances such as Provisc (13). More effective isolatory layer of Viscoat on the corneal endothelium may be attributable to the fact of its chemical structure. Due to chondroitin sulfate component Viscoat has three negative charges per molecular unit, which provides greater neutralizing effect on positively charged ocular tissue, which explains better coatability and thicker layer on corneal endothelium (14). All these data can potentially explain significantly lower mean rate of endothelial cell loss after surgery in the Viscoat group. These data can support previous reports that dispersive viscoelastic substances as well as low molecular weight cohesive agents protect endothelial cells more effectively particularly in hard nucleus phacoemulsification (15). In contrast, lack of electrical charge of hydroxypropyl methylcellulose and only one negative charge of more cohesive Provisc is the reason of lower adherence tendency and lower level of protection against the increase of temperature (16). On the other hand, we found significantly higher MLT in soft shell technique group as compared with Viscoat. It seems reasonable that sequential injection of cohesive-dispersive viscoelastic substances without mixing made the aspiration of the cohesive part of viscoelastic substances rapid, leaving relatively thinner layer of dispersive viscoelastic substances retained and adherent to the endothelium. However, the well known fact of faster aspiration of cohesive viscoelastic substances from anterior chamber and no difference in maximal temperature between Provisc and soft shell technique is not clearly understood. The results may suggest that adherence of low amount of hyaluronates to their hyaluronic acid binding sites on corneal endothelium is sufficient for protection against lower level of temperature (17). Based on aforementioned thermographic observations, it seems reasonable that the highest MLT was measured for 22 °C BSS. Furthermore, the use of cooled irrigating solutions without additional filling of anterior chamber with viscoelastic substances allowed lowering of the maximal temperature to the level comparable with Viscoat. Mean rate of corneal endothelial cells loss in the group of cold BSS was statistically lower as compared with the group in which normal temperature of BSS was used and was similar to Provisc group. This information is of clinical importance, implying that cooling of irrigating solutions or viscoelastic substances during phacoemulsification may serve as an additional protection method against intraoperative thermal injury. These results support previous observations of Findl et al regarding cooled irrigating solution for reduction of blood-aqueous barrier breakdown and postoperative inflammation, which are also in part the effect of thermal injury (8).

Analysis of the TMTA, which in fact represents temperature transmission property of different substances, showed significant difference among all the examined groups. Shorter TMTA were obtained for the normal temperature and cold BSS groups, respectively, which reflects longer exposure to maximal level of the temperature. In contrast, this study's measurements showed significantly longer TMTA among viscoelastic substances groups. The fact that Celoftal was found to have the longest TMTA is most likely explained by synthetic composition and dispersive behavior of hydroxypropyl methylcellulose, which potentially allows for slower temperature transmission as compared with naturally originated viscoelastic substances. Although significantly lower values of the rate of endothelial cell loss were assessed in Viscoat, the values obtained in other groups showed correlation to the TMTA rather than to MLT. This finding supports other studies indicating that cell loss is minimized when dispersive viscoelastic substances are used (18), therefore this may suggest that rapid rise of temperature may have relevant impact partially contributing to endothelial cell damage.

In conclusion, our study showed that currently used viscoelastic substances give different levels of corneal endothelial cell protection against the increase of temperature during phacoemulsification.

This fact is mostly attributable to some physicochemical properties of viscoelastic substances, e.g., retention and coatability. Our study suggests that dispersive viscoelastic substances are the most effective in protecting corneal endothelial cells against thermal injury. Moreover, our observations indicate that cold irrigating solutions may lower the level of maximal temperature; however, a sharp significant rise of temperature that follows the use of ultrasound energy may potentially affect the damage of corneal endothelial cells.

Surgical strategy during phacoemulsification should also consider the choice of the most adequate viscoelastic substances or chilled irrigating solutions from the point of view of the most effective protection against the increase of temperature, particularly in difficult surgical cases, e.g., hard nucleus, shallow anterior chamber, and primary endothelial abnormalities. Additional physical studies of thermal properties of viscoelastic substances are required to better understand the unique benefits and limitations of each of these currently used viscoelastic solutions.

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Reprint requests:
Piotr Jurowski, MD
Mimozy 35 A
91- 864 Lodz, Poland
p.jurowski@poczta.wprost.pl

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