Protection of corneal endothelium from irrigation damage: a comparison of sodium hyaluronate and hydroxypropylmethylcellulose

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Abstract

Purpose To compare the endothelial protection of sodium hyaluronate and hydroxypropylmethylcellulose against endothelial damage induced by irrigation.

Methods An *in vitro* assay with freshly excised porcine eyes was developed using the Janus green photometry technique. Irrigation and aspiration technique was standardised. Forty pairs of porcine eyes were used. One randomly chosen eye was filled with sodium hyaluronate (SH) and the other with hydroxypropylmethylcellulose (HPMC). Irrigation and aspiration was carried out with balanced salt solution for 5 min. Twenty additional pairs of porcine eyes served as controls. Student's *t*-test was used for statistical analysis.

Results Both viscoelastic agents protected the endothelium as compared with controls. The endothelial protection, determined with the Janus green photometric technique, was significantly greater with HPMC than with SH.

Conclusions Viscoelastic agents are effective in protecting the endothelium from irrigation damage in porcine eyes *in vitro*. HPMC provided greater protection than SH in this particular model.

Key words Cataract, Cornea, Endothelium, Irrigation-aspiration, Janus green, Viscoelastics

One of the most important functions of viscoelastic solutions in ophthalmology is protection of the corneal endothelium during cataract surgery. The corneal endothelium can be accidentally damaged by instrument or intraocular lens touch, by lens fragments and air bubbles during phacoemulsification and by irrigating solutions used during routine irrigation and aspiration.¹⁻³ All the viscoelastic

agents presently in use possess sufficient viscosity at rest to provide a mechanical barrier from intraocular lens and instrument touch of the endothelium. Hyaluronic acid based viscoelastics, such as sodium hyaluronate of high molecular weight (SH; Healon, Pharmacia) and sodium hyaluronate of lower molecular weight (Amvisc Plus, Iolab) provide protection under these circumstances because of their high viscosity at rest.³⁻⁶ With increased emphasis on the removal of cataract by phacoemulsification, the potential for damage from air bubbles, lens fragments and irrigating solutions is increased. This shift in technique has emphasised the endothelial coating properties of viscoelastic solutions.

From a clinical standpoint viscoelastic agents can be divided into two broad categories relating to gross endothelial viscoelastic coating. SH is an example of the first category, which do not provide a visible endothelial coating during surgery and are easily aspirated from the anterior chamber. In the second category, viscoelastics such as hydroxypropylmethylcellulose (HPMC; Occucoat, Storz) and chondroitin sulphate-sodium hyaluronate (Viscoat, Alcon) produce a visible endothelial coating and are less easily aspirated.⁷

Studies have documented, at least in the short post-operative period, greater endothelial protection from solutions producing grossly visible endothelial coatings during ultrasonic agitation of the anterior chamber with air bubbles.³ It has also been noted that irrigating solutions can cause endothelial damage.⁸

In this study we describe the use of the Janus green photometry technique⁹ to quantify the difference in endothelial damage occurring during irrigation and aspiration (I&A) of the anterior chamber of porcine eyes with and without HPMC or SH.

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Materials and methods

Janus green standard curve

The Janus green photometric technique was used to assess endothelial damage.⁹ Twenty serial dilutions of Janus green (Sigma) ranging from 0.5 mg/l to 20 mg/l were made in absolute alcohol. Samples corresponding to each concentration were read in a photometer at 650 nm. Extinction values of each sample were plotted against their respective concentrations to give the Janus green standard curve

Janus green staining and elution

Porcine eyes were used in this study. Freshly excised pig eyes from 9- to 12-month-old animals were obtained from an abattoir (Hatfield Meat Packing Co.). Each pair of eyes was placed in a separate bottle and transported on ice. The eyes were processed within 60–150 min of enucleation. Corneoscleral buttons with a 2 mm scleral rim were excised, taking care not to damage the endothelium. All excisions were done by the same investigator (H.S.D.). The corneoscleral buttons were rinsed in a sterile balanced salt solution (BSS, Alcon Laboratories, Fort Worth, TX) containing 0.05% Tween-20, for 3 min. They were then placed in a shallow wax cup with the epithelial surface against the wax. The endothelial surface was covered with 200 µl of a 1% solution of Janus green in BSS for 90 s. Excess dye was tipped off and the corneoscleral button was rinsed in

three changes of BSS for a total duration of 3 min. A 9 mm button was punched out from the centre of the cornea using a punch trephine (H&I Instrumentation, NJ). The corneal button was placed in a test tube containing 1 ml of absolute alcohol for 90 s to elute dye bound to the corneal button. The tube was vortexed and the extinction value of the solution read at 650 nm.

Standard endothelial damage

Fifteen pairs of porcine eyes were processed to calculate Janus green extinction values for standard endothelial damage. The corneoscleral button obtained from one eye of each pair was directly stained with Janus green. Extinction values of this group were taken to represent '0.0%' endothelial damage. Endothelium from the corneoscleral buttons obtained from the other eye of each pair was mechanically wiped off with moist cotton and then stained with Janus green. Extinction values of this group were taken to represent '100%' endothelial damage. The extinction values corresponding to '0.0%' and '100%' damage were superimposed on the Janus green standard curve. Intermediate points corresponding to '25%', '50%' and '75%' damage were also marked (Fig. 1).

Corneoscleral buttons obtained from a further 20 paired porcine eyes were subjected to '0.0%' and '100%' damage as described above. Each of 10 pairs of corneoscleral buttons was covered with SH for 5 min and the remaining 10 pairs covered with HPMC for 5 min.

Fig. 1. Janus green photometry of corneoscleral buttons that were subjected to '0.0%' and '100%' endothelial damage prior to staining (mean values and standard deviation). Controls were corneoscleral buttons that were not pre-treated with SH (Healon) or HPMC (Occucoat). The Healon and Occucoat columns represent corneoscleral buttons that were pre-treated with Healon or Occucoat respectively for 5 min before staining.

One corneoscleral button of each pair had '0.0%' endothelial damage and the other had '100%' endothelial damage. At the end of 5 min the corneoscleral buttons were processed for Janus green staining and elution as described above.

Standardisation of irrigation and aspiration for optimal endothelial damage

Porcine eyes were mounted on a stand and held in a clamp under a stereomicroscope. The clamp was tightened sufficiently to give an intraocular pressure of 15–18 mmHg. A standard 3 mm limbal incision was made and the anterior chamber irrigated and aspirated with BSS using a Cavitron/Kelman I&A unit (Cooper Vision, Irvine, CA) with the switch in the 'IA min' position, giving a flow rate of approximately 25 ml/min. The tip of the I&A canula was kept in the centre of the pupil in the plane of the iris with the irrigation ports directed horizontally. The anterior chambers were irrigated and aspirated for 3, 5 and 8 min. Ten pairs of eyes were used for each time point. One eye of each pair was subjected to I&A while the other served as control. After I&A, corneoscleral buttons were dissected and processed for Janus green staining and elution as described.

Comparison of the protective effect of SH and HPMC against I&A-induced endothelial damage

Forty pairs of eyes were used in this experiment. The anterior chamber of one eye of each pair, right or left picked at random, was filled with 0.25–0.3 ml of SH and the other was filled with a similar volume of HPMC.

Irrigation and aspiration with BSS was carried out for 5 min as described. The corneoscleral buttons were dissected. SH was more readily rinsed off the corneoscleral buttons than HPMC when BSS was used as the rinsing solution but both washed off equally well when 0.05% Tween-20 was added to the BSS solution and the buttons rinsed for 3 min before Janus green staining. The corneal buttons were then processed for Janus green staining and elution as described above. Addition of Tween-20 to the wash solution and rinsing for 3 min did not induce any further damage to the endothelium as assessed by Janus green staining. This was determined in pig eyes before including the use of Tween-20 in the experiment.

Results

The extinction values of the different concentrations of Janus green in absolute alcohol were plotted on a graph. The extinction values showed a progressive linear correlation with the concentration of Janus green. The mean value for '0.0%' corneal endothelial damage was 0.04 and that for '100%' damage was 1.17 (Fig. 1).

Control corneoscleral buttons in which '0.0%' or '100%' endothelial damage was produced and the surface covered with SH or HPMC for 5 min before Janus green staining and elution gave extinction values very similar to those where these substances were not used (Fig. 1).

Three minutes of I&A with BSS produced an average of 19.6% corneal endothelial damage, 5 min of I&A produced an average of 43.5% corneal endothelial damage and 8 min of I&A produced an average of 49.5% corneal endothelial damage (Fig. 2). The mean Janus

Fig. 2. Janus green photometry values (mean and standard deviation) following staining and elution of corneoscleral buttons from eyes that were subjected to irrigation and aspiration with balanced salt solution for 3, 5 or 8 min before staining.

Fig. 3. Janus green photometry values (mean and standard deviation) following staining and elution of corneoscleral buttons from eyes where the anterior chamber was filled with SH (Healon) or HPMC (Occucoat) or neither (controls) prior to irrigation and aspiration for 5 min. Healon and Occucoat were used in paired right and left eyes of the same animals.

green photometric value of the 40 corneas protected with SH was 0.366, corresponding to an average of 30.7% endothelial damage. The mean Janus green photometric value of the 40 corneas protected with HPMC was 0.24, corresponding to an average of 20.5% endothelial damage. Both SH ($p < 0.01$, Student's *t*-test) and HPMC ($p < 0.001$, Student's *t*-test) significantly protected the corneal endothelium from I&A-induced damage compared with controls (Fig. 3). Of the two substances tested, HPMC afforded better protection to the corneal endothelium than SH ($p < 0.001$, Student's paired *t*-test).

Discussion

In the Janus green photometric technique the permeability of the cell and nuclear membranes for the vital stain Janus green is used as a marker for cell damage and cell death. This technique is simple and easy to use, independent of the user's experience and requires only standard laboratory equipment (photomicroscope, spectrophotometer).⁹⁻¹² Pharmakakis *et al.*¹⁰ demonstrated that the Janus green photometric technique is an accurate method for determining endothelial damage, and can successfully replace the method of cell counting to evaluate the percentage of damaged endothelial cells.

This study established that I&A can produce significant damage to the corneal endothelium of enucleated porcine eyes processed within 150 min of enucleation. The amount of endothelial damage as assessed by the Janus green photometry technique seems unduly high for the 5 min of I&A but could be due the time interval of 60–150 min between enucleation and

performing the test. The fact that these were post-mortem eyes that were transported on ice (over a short distance) and brought to room temperature for the study, could have stressed the endothelium. Further, all eyes were hypotonus post-mortem and had to be clamped to bring the pressure up to 15–18 mmHg. This could also have contributed to the endothelial stress. These factors may have rendered the endothelium more vulnerable to irrigation-induced damage and could account for the high degree of endothelial damage observed in this study. It is possible that Tween-20 may have induced some degree of further endothelial damage, following I&A, but this was not found to be significant. Any damage induced by the wash solutions or procedure would be applicable to both test groups and controls. Since right and left eyes of the same animal were used for comparison and as controls the comparative protection afforded by the two agents tested is still significant. Both SH and HPMC protected the endothelium against I&A damage but HPMC was significantly more effective. It is of interest to note that, compared with SH, HPMC was more difficult to rinse from the corneal endothelial surface. In order to make a valid comparison between SH and HPMC, Tween-20 had to be added to the rinsing solution (BSS) and the rinsing time increased to 3 min instead of the 2 min described in the original procedure.⁹ The affinity of HPMC for the endothelial surface is most likely responsible for the greater protective effect observed in this study.

The mechanism of endothelial protection by viscoelastic solutions is poorly understood. While solutions such as HPMC and chondroitin sulphate–sodium hyaluronate produce a clinically visible

endothelial coating which protects the cornea in the short term post-operatively, it is not clear, in the long term, whether the presence of this coating or the particular molecules themselves alter the endothelial microenvironment.

The endothelium has known hyaluronic acid receptors as well as a normal coating of hyaluronic acid molecules which is easily washed away by endothelial irrigation.^{13,14} Interestingly, studies have shown that the endothelial coating of hyaluronic acid can be replaced by similar molecules placed in the anterior chamber.¹³ The significance of the normal hyaluronic coating to the endothelium and its health is uncertain.

The clinical relevance of this study is not known as it is difficult to extrapolate data obtained from post-mortem eyes to the viable clinical situation. Simple irrigation for a brief period of time usually does not appear to cause endothelial damage in human endothelium *in vivo*. This communication has demonstrated the beneficial effect of viscoelastic agents in protecting the endothelium from irrigation damage in porcine eyes *in vitro*. Further research is needed to investigate the potential clinical importance of these findings.

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