Water-mediated lysis of lens epithelial cells attached to lens capsule

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Purpose: To investigate the effect of distilled deionized water (DDW) on lens epithelial cells (LECs) attached to the lens capsule.

Setting: Wound Healing Research Laboratory, Center for Vision Research, Westmead Hospital, Sydney, NSW, Australia.

Methods: Anterior capsulotomy specimens taken during routine cataract surgery were divided in half. One half was immersed in DDW and the other half in culture medium (control) for 1 to 5 minutes and photographed at intervals by phase-contrast microscopy. In further experiments, the capsules were exposed to DDW for 1 or 2 minutes and placed in culture for 1 week to determine whether LECs survive treatment and are capable of repopulating the lens capsule.

Results: Distilled-deionized water induced marked swelling of the cytoplasm within 60 seconds of treatment. At 120 seconds, there was disruption of the plasma membranes, with few intact cells remaining. In the control capsules, confluent monolayers of LECs covered the entire capsule surface with a halo of LECs growing on the surrounding plastic well. Viable LECs were observed in 1 of 3 capsules treated for 1 minute with DDW. These did not reach confluence or grow off the capsule onto the surrounding well. No viable LECs were seen on capsules exposed to DDW for 2 minutes.

Conclusions: Short exposure of LECs to DDW induced extensive and rapid cell lysis. Distilled-deonized water may be a useful agent for instillation in the capsular bag during sealed-capsule irrigation to prevent posterior capsule opacification.

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Posterior capsule opacification (PCO) is the most common cause of reduced vision after cataract surgery[1] and remains a significant cause of visual handicap in patients who do not have access to laser capsulotomy. It is a major hurdle in the successful visual rehabilitation of the pediatric population after cataract surgery, with a 4-year incidence of up to 39%.[2] It also limits advances in the development of intraocular lenses (IOLs), particularly accommodating IOLs.[3]

Sealed-capsule irrigation (SCI) is a surgical technique that permits irrigation of the capsular bag after the cataractous lens is removed while maintaining mechanical isolation from the rest of the eye. The technique allows delivery of pharmacologic agents into the capsular bag during surgery without the attendant risk for collateral damage to other ocular tissues.[4] To target lens epithelial cells (LECs), several cytotoxic agents have been investigated in human[5] and animal studies,[6] ex vivo capsular bag organ culture,[7,8] and LEC cultures in vitro.[9–12] Recent evidence, however, suggests that the lens capsule protects LECs from the effects of cytotoxic drugs such as staurosporine by an unidentified mechanism (F. Tholozan, ARVO abstract 1228, 2003). This,

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together with other risks in using cytotoxic drugs, led us to look for alternative agents to eliminate LECs.

Distilled-deionized water (DDW) induces cell lysis by osmotic stress[13]; it is inexpensive, safe to handle, and rapidly neutralized by the instillation of balanced salt solution. We have recently provided data to demonstrate that LECs maintained in culture for 1 week are lysed by exposure to DDW.[14] The aims of this study were to investigate the morphological changes observed in cultured and freshly isolated LECs after exposure to DDW and to determine whether LECs treated with DDW are able to repopulate the lens capsule in culture.

Materials and Methods

Preparation of Capsulotomy Specimens

Capsulotomy specimens of approximately 5.0 mm were obtained during routine cataract surgery. The capsulorhexis was performed using trypan blue 0.06% (Vision Blue®) to optimize visualization for subsequent handling outside the eye. Informed consent was obtained and local ethics approval granted. The specimens were gently rinsed with balanced salt solution and split in half; each half was attached with entomology pins to separate wells of a 6-well culture dish (Becton Dickenson). The capsules were treated in 2 arms: (1) Freshly isolated capsules were exposed to DDW within 10 minutes of removal from the eye or (2) cultured capsules that had been maintained in cell culture conditions were exposed to DDW 1 week after removal from the eye.

Capsule Culture

Capsulotomy specimens were obtained immediately during surgery, transfered to sterile culture dishes, and incubated in RPMI-1640 culture medium with L-glutamine; 500 mL of the medium was supplemented with penicillin/streptomycin (25 000 units), amphotericin (250 units), and 10% fetal bovine serum (all Gibco-BRL). After 1 week in culture, visual inspection under phase-contrast microscopy confirmed the LECs had formed a confluent monolayer on the lens capsule with a 360-degree halo of cells growing off the capsule onto the plastic culture dish.

Distilled-Deionized Water Treatment

Balanced salt solution (fresh capsule treatment) or RPMI culture medium (culture capsule treatment) was aspirated and replaced with 2.5 mL of DDW (or fresh RPMI for controls). After treatment, the DDW was aspirated and quickly replaced with fresh culture medium. The specimens were examined under a phase-contrast microscope (Leica) and photographed with a digital camera (Coolpix 950, Nikon) attached via a Leica camera adapter.

Results

Time Course of DDW Treatment in Cultured Capsulotomy Specimens

Within 60 seconds of exposure to DDW, the LECs were markedly swollen with intact plasma membranes. At 90 seconds, there was clear disruption of the plasma membrane in many LECs. By 120 seconds, no intact cells were visible. Although LEC morphology was clearly discernible in LECs attached to the plastic culture dish, the high concentration of LECs on the lens capsule precluded accurate examination of the plasma membranes. Figure 1 shows a representative experiment of 3 separate capsules treated independently.

Fresh Capsules Treated Immediately After Removal from the Eye

The experiments with the fresh capsules were performed to ensure that LECs at the time of surgery had the same susceptibility as LECs maintained in culture for 1 week before treatment. They also provided better visualization of the morphology of LECs attached to the capsule surface. The halved capsulotomy specimens were treated within 10 minutes of being removed from the eye. One half was treated with DDW for up to 180 seconds; the other half was treated with RPMI for the same time to serve as a control. After 60 seconds, there was marked cell distention with occasional cell lysis. At 120 seconds, there was significant damage to plasma membranes and after 180 seconds, all LECs appeared lysed (Figure 2). Confluent monolayers of intact LECs were seen in the control, RPMI-treated specimens.

Viability of LECs 1-Week Post Treatment

To determine whether LECs exposed to 60 or 120 seconds of DDW were capable of recovering to proliferate and repopulate the lens capsule, specimen pairs were cultured for 1 week after treatment. Specimen pairs were included in the analysis if the control half of the capsule exhibited a confluent monolayer with a 360-degree halo of LECs growing onto plastic. Unless this was the case, it would not be possible to attribute cell loss in the treatment group to the treatment itself. Thus, specimen pairs in which the control half had scant or no coverage of LECs were not included.

Six of 9 control specimens fulfilled the inclusion criteria. The matched pairs had been treated for 60 sec-

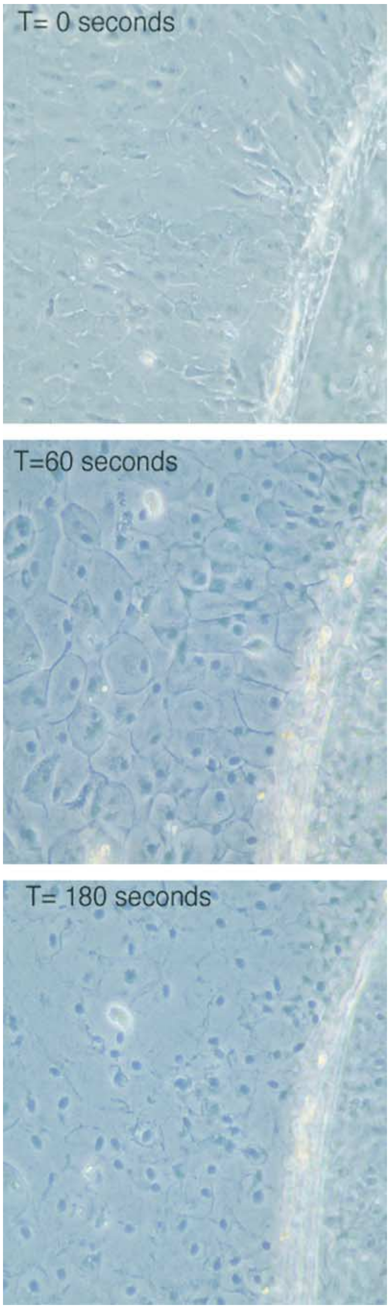

Figure 1. (Crowston) The effect of DDW on cultured primary LECs. A human lens capsule with a curved edge on the far right of the field supports a confluent monolayer of LECs. Additional LECs to the left of the capsule edge are growing off the capsule onto the surrounding culture dish. Specimens (n = 3) were exposed to DDW and photographed under the phase-contrast microscope (original magnification ×20) at the times shown. Gross swelling of LECs with minimal cell lysis was observed at 60 seconds and significant cell lysis by 90 seconds. Most cells were lysed by 120 seconds.

onds (n = 3) or 120 seconds (n = 3). After 1 week in culture, capsule specimens were exposed to trypan blue (0.4%) for 1 minute and rinsed in culture medium immediately before being photographed. Capsulotomy specimens that had been treated with DDW for 120 seconds had no intact LECs attached to the capsule or surrounding plastic (Figure 3). One of 3 capsules treated with DDW for 60 seconds had viable cells on the capsule surface that excluded trypan blue. These were maintained in culture for a second week but did not reach confluence and did not grow onto the surrounding plastic of the well. The remaining 2 specimens had no viable cells on the capsule surface.

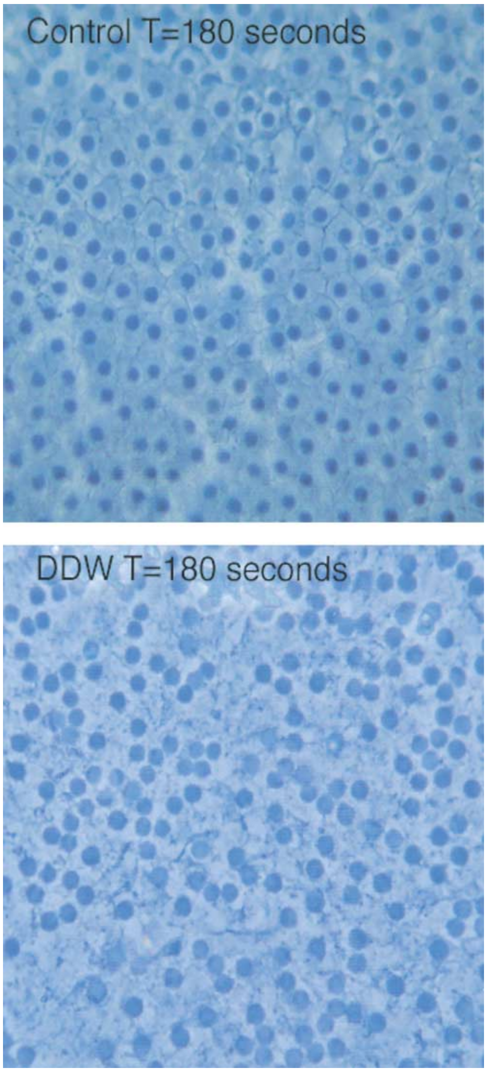

Figure 2. (Crowston) The effect of DDW on capsulotomy specimens ex vivo. Specimen halves were exposed within minutes of removal from the eye to RPMI culture medium (*top*) or DDW (*bottom*) for 60 or 120 seconds. Samples were then immersed in RPMI culture medium and photographed immediately at ×40 magnification using phase-contrast microscopy. Plasma membranes appear intact in the control sample. There is extensive disruption of plasma membranes in the water-treated capsule.

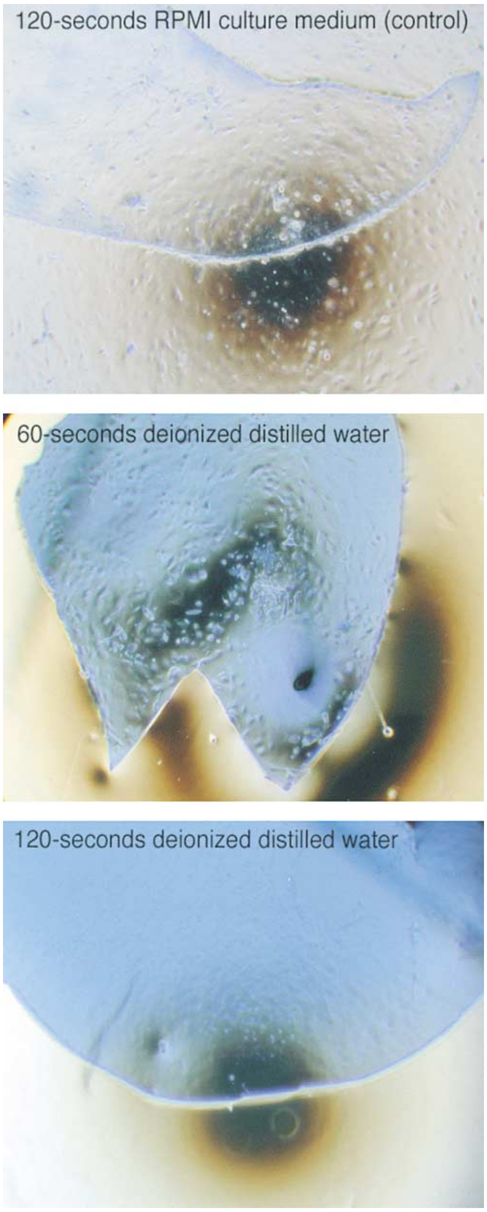

Figure 3. (Crowston) Capsulotomy specimens cultured for 1 week after treatment with DDW. Capsulotomy specimen pairs (n = 3 for each group) were placed in culture for 1 week after treatment with RPMI culture medium (120 seconds) (*top*), DDW for 60 seconds (*middle*), or DDW for 120 seconds (*bottom*). A confluent monolayer of LECs covers the capsule and extends onto the surrounding culture dish in all the control capsules. Viable LECs are seen on 1 of the 3 capsules treated for 1 minute (*middle*). However, these are relatively sparse and do not grow onto the surrounding culture dish. No viable cells were observed on the capsule treated for 2 minutes (*bottom*). Capsules were immersed in trypan blue 0.5% for 1 minute before photography (original magnification ×10 objective).

Discussion

In this study, DDW had a dramatic effect on LECs, with rapid cell lysis occurring after approximately 90 seconds. This model does not lend itself to quantitative analysis because of inevitable differences in the starting cell number on the capsulotomy specimens. A dual-stain, live/dead assay could be used for cells treated for 60 seconds; however, the absence of intact cells after 120 seconds obviates the value of such an assay for this treatment group. The advantage of this model is that primary human LECs can be treated immediately after removal from the eye and maintained on their physiological substrate, the lens capsule. Furthermore, the ability to place treated capsules in culture permits evaluation of whether the LECs recover to repopulate the lens capsule, which in our view provides a more meaningful end point than proliferation rates or live/dead ratios. Observations that trypan blue vital dye is excluded from LECs in the control capsules and the single capsule specimen with LECs after 1 minute of DDW confirm that these cells are viable. This suggests that LECs may have the potential to survive after 1-minute exposure to DDW. Whether these cells have the capacity to fully repopulate the lens capsule and ultimately induce PCO requires further investigation.

Human LECs can be propagated in vitro; however, these cells require high seeding density and in our hands can be propagated only for low passage numbers. Although transformed LEC lines are readily available, their use and the relevance of findings, particularly their response to toxic agents, should be assessed cautiously. We opted to treat ex vivo human capsulotomy specimens containing live LECs as this most closely resembles the clinical situation. When placed in culture, these cells proliferate rapidly on the capsule, providing an in vitro model of aggressive PCO.[15] The lens capsule did not appear to protect LECs from DDW-mediated lysis. This differs from the apparent protection afforded by the lens capsule that appears to make LECs resistant to many cytotoxic agents.[12]

An inescapable problem of previous attempts to use cytotoxic agents to eliminate PCO has been collateral damage to other parts of the eye. Sealed-capsule irrigation permits isolation of the capsular content from the rest of the eye. Recent data from rabbit studies show that reliable resealing of the capsule is feasible with no evidence of collateral damage after prolonged intracapsular irrigation with mitomycin-C, distilled water, or the detergent triton-X (A. Maloof, ARVO abstract 284, 2003). These studies suggest not only that the capsule can be reliably sealed but also that these agents do not pass freely through the capsule in the rabbit.

The qualitative data in our study indicate that DDW induces rapid lysis of human LECs ex vivo and in cultured lens capsules. The lens capsule does not appear to confer significant protection to LECs from exposure to DDW, as it may do after exposure to certain cytotoxic drugs. Distilled-deionized water has several clear advantages as a treatment for PCO. It is inexpensive, safe to handle, reversible with balanced salt solution, and readily available. We propose that DDW merits further investigation as a candidate for infusion into the capsular bag with sealed-capsule irrigation.

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