

Transmission electron microscopy of the rabbit posterior capsule irrigated with thapsigargin and 5-fluorouracil in a sealed-capsule irrigation device

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Abstract

Purpose To investigate, by transmission electron microscopy (TEM), the effect on the posterior capsule of a young rabbit eye of 5-fluorouracil (5-FU) or thapsigargin in a sealed-capsule irrigation device.

Setting St Erik's Eye Hospital, Stockholm, Sweden.

Methods Clear lens extraction was performed unilaterally in eight 4-week-old rabbits. A sealed-capsule irrigation device was irrigated for 2 min with 20 ml of one of the following: balanced salt solution (BSS; $n = 2$), thapsigargin 300 μM ($n = 2$), 5-FU 50 mg/ml ($n = 2$), or 5-FU 25 mg/ml ($n = 2$). The substances were washed out for 10 s with BSS. The eyes were left aphakic. Six weeks postoperatively, the animals were killed, and the posterior capsule was extracted and fixed for TEM. As a control, we also evaluated the capsules from the two fellow eyes in the BSS group that did not undergo surgery.

Results The ultrastructure of the posterior capsule in eyes irrigated with 5-FU or thapsigargin did not differ from that in the eyes irrigated with BSS or in the eyes that did not have surgery. The membranes had the same ultrastructure with thin collagen fibres on the anterior and posterior face of the posterior capsule and an amorphous matrix.

Conclusion Thapsigargin or 5-FU used in a sealed-capsule irrigation device does not seem to harm the posterior capsule, which appeared similar to when the capsule is irrigated with BSS.

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Introduction

Preventing posterior capsule opacification (PCO) plays an important role in improving visual acuity after cataract surgery in adults and children.^{1,2} Recently, the use of antiproliferative drugs has been studied as a successful modality to target lens epithelial cells (LECs).^{3–6} The sealed-capsule irrigation device (SCI) Perfect Capsule^{7,8} (Milvella Ltd, Epping, Australia) was reported to be highly efficacious in sealing the capsular bag from the adjacent ocular structures in rabbit eyes.³ In humans, Rabsilber *et al.*,⁹ reported that the device was safe when used after phacoemulsification. The capsular bag then was flushed with distilled deionized water (DDW), and afterwards a hydrophilic intraocular lens (IOL) was implanted. Irrigation with DDW, however, did not prevent the development of PCO either in humans⁹ or rabbits.³

We observed a beneficial effect in preventing PCO when the sealed system was irrigated with 5-fluorouracil (5-FU) 50 mg/ml in an animal model.³ We also reported the safety of 5-FU 50 mg/ml when used with the Perfect Capsule

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with no harmful effects to the surrounding tissues, such as the corneal endothelium, trabecular meshwork, and retina in young rabbits.¹⁰ We also evaluated a lower concentration of 5-FU (25 mg/ml), but this concentration did not have the same beneficial effect.⁴ Irrigation with thapsigargin also did not have a good effect in rabbit eyes;⁴ however, thapsigargin used *in vitro* in human capsules did have a beneficial effect.⁵

It is important to ascertain that the capsular bag is not damaged by either the use of an antiproliferative drug or by the absence of LECs. In this study of rabbit eyes, we used transmission electron microscopy (TEM) to study the posterior capsule morphology after exposure to different irrigation substances in the SCI system.

Materials and methods

Animals

Eight New Zealand 4-week-old white rabbits (average weight, 315 g) underwent unilateral clear lens extraction. The experiment was approved by the Northern Stockholm Animal Experiments Ethics Committee and adhered to the Association for Research in Vision and Ophthalmology statement on the use of animals in ophthalmic and vision research.

Surgery

Surgery was performed by one experienced anterior segment surgeon after general anaesthesia was induced as described previously.³ Briefly, a 2.8-mm clear corneal incision and small capsulorrhexis were created. Clear lens extraction using irrigation/aspiration was performed and the Perfect Capsule was inserted and sealed to the anterior capsule. The capsule was flushed with one of four substances for 2 min and balanced saline solution (BSS) for 10 s to wash out the residual substance, and the incision was closed with a 10-0 nylon suture. Cefuroxime (1 mg) was injected intracamerally at the end of surgery.

Study design

Two eyes each were irrigated with either BSS, thapsigargin 300 μ M (Sigma), 5-FU 50 mg/ml, or 5-FU 25 mg/ml. About 20 ml of each substance was washed through the system during the 2-min irrigation period.

All animals were sacrificed 6 weeks after surgery. The operated eye was enucleated and the capsular bag was dissected and immediately placed in fixative. To compare the capsules of the operated eyes with normal non-operated phakic eyes, we also extracted the capsular bags including the crystalline lens of the two fellow eyes in the rabbits irrigated with BSS.

TEM

The capsular bag was dissected and small pieces were fixed in 2% glutaraldehyde and 0.5% paraformaldehyde in 0.1 M sodium cacodylate buffer containing 0.1 M sucrose and 3 mM CaCl₂ (pH 7.4) at room temperature for 30 min followed by 24 h at 4°C. Specimens were rinsed in 0.15 M sodium cacodylate buffer containing 3 mM CaCl₂ (pH 7.4), postfixed in 2% osmium tetroxide in 0.07 M sodium cacodylate buffer containing 1.5 mM CaCl₂ (pH 7.4) at 4°C for 2 h, dehydrated in ethanol followed by acetone and embedded in LX-112 (Ladd, Burlington, VT, USA). Semi-thin sections were cut and stained with toluidine blue and used for light microscopic analysis. Ultrathin sections (approximately 40–50 nm) were cut and contrasted with uranyl acetate followed by lead citrate and examined in a Tecnai 10 transmission electron microscope at 80 kV. Digital images were taken by using a Mega View III digital camera (Soft Imaging System, GmbH, Münster, Germany).¹¹

Results

TEM showed an ultrastructure of thin collagen fibres on the anterior and posterior part of the posterior capsule. The collagen fibres are thin parallel lines that comprise approximately one-third of the thickness of the membrane. The rest of the membrane is embedded in an amorphous matrix. The ultrastructure was similar in all eyes, and no damage was observed in the eyes irrigated with BSS, 5-FU, or thapsigargin (Figure 1).

The specimens from the non-operated eyes were more difficult to analyse, probably because they included the crystalline lens, which makes sectioning difficult. However, morphologic analysis of the capsules showed the same ultrastructure compared with the operated eyes (Figure 2).

Discussion

This study showed no morphologic changes in the posterior capsule after irrigation with either thapsigargin or 5-FU. It is important to determine whether the capsule is damaged by different drugs or an absence of cells, since the capsule might rupture and the IOL could dislocate. The optimal substance and irrigation time associated with the Perfect Capsule have been debated and studied extensively. The substance that initially was intended to be used, DDW, was ineffective in humans or rabbits.^{3,9} Mitomycin C or 5-FU has been reported to inhibit the proliferation of LECs and prevent PCO.^{3,6} However, the use of these agents may be toxic to the ocular tissues. In our previous study of the safety of 5-FU 50 mg/ml, the adjacent ocular tissues were undamaged

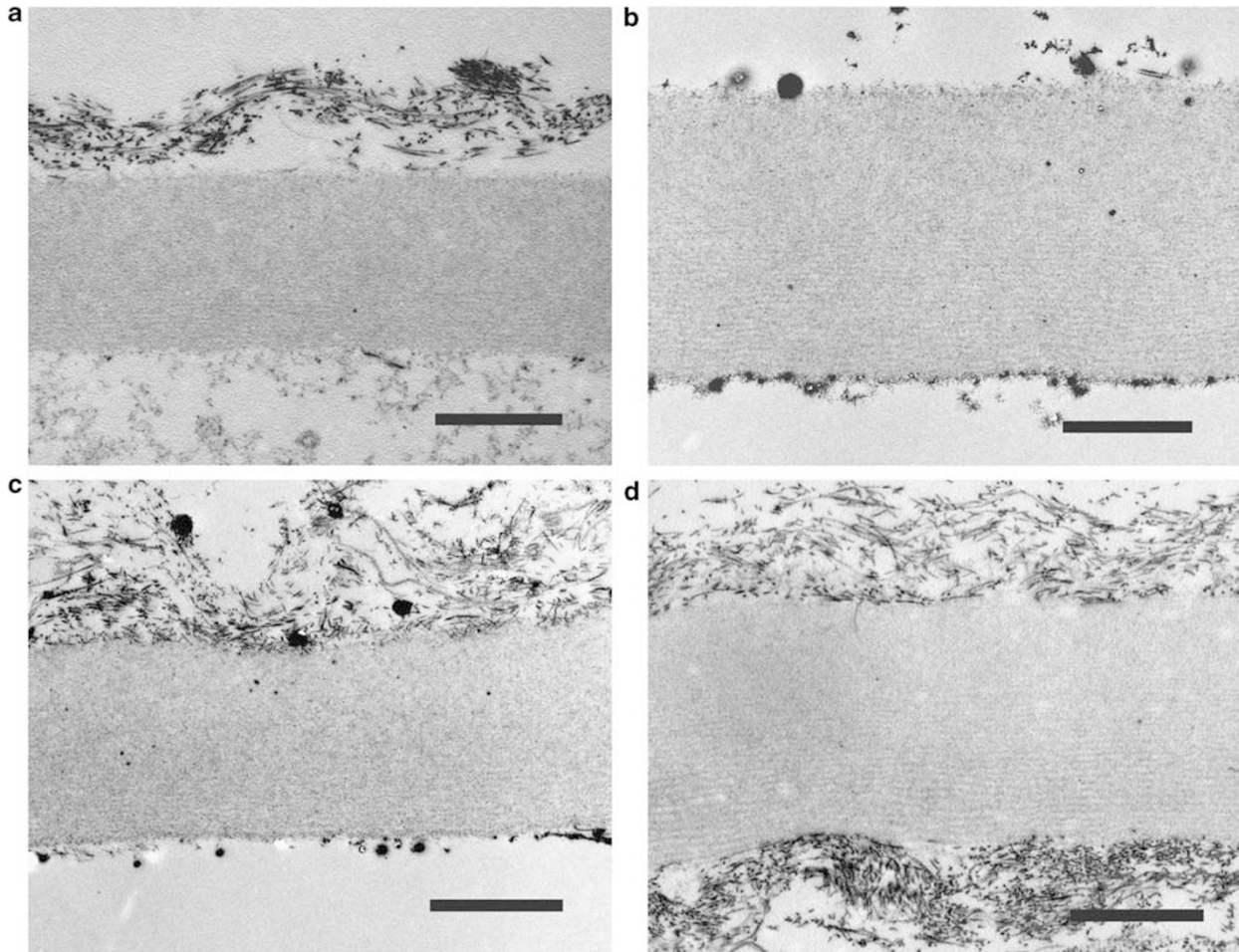


Figure 1 TEM image of part of the posterior capsule in eyes in which the sealed-capsule irrigation device was irrigated with (a) BSS, (b) thapsigargin 300 μM , (c) 5-FU 50 mg/ml, or (d) 5-FU 25 mg/ml. Bars denote 2 μm .

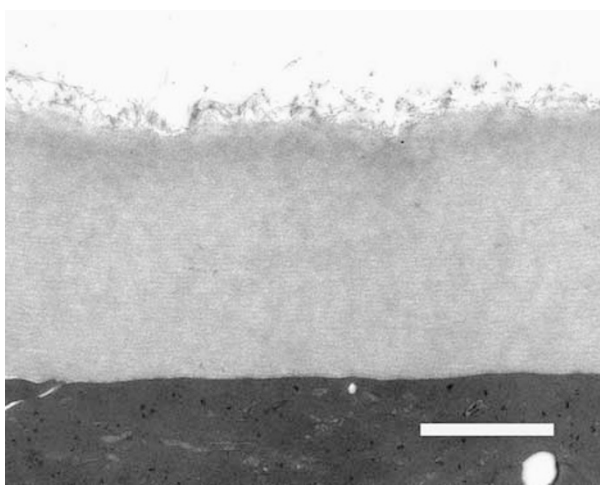


Figure 2 TEM image of a part of the posterior capsule in an eye that did not undergo surgery. Bar denotes 2 μm .

even when 5-FU was injected into the bag with the Perfect Capsule and left for 2 min before aspiration and irrigation.¹⁰ The previously cited study of mitomycin C with the Perfect Capsule reported that the drug was safe and did not damage the anterior lens capsule and central corneal endothelium, but its effect on the posterior capsule needs to be tested. In addition, if leakage occurs, it is uncertain whether mitomycin C is safe, because in that study, Perfect Capsule was used in all eyes.⁶ Mitomycin C and colchicine use in other studies had been reported to inhibit the growth of LEC.^{12,13} Mitomycin C also was reported to damage cultured corneal keratocytes and cause bullous keratopathy in patients with glaucoma.^{14,15} The safety of mitomycin C is questionable.

SCI increases the potential use of antimetabolites to target the LECs after cataract surgery. TEM has been used

earlier to evaluate the lens and capsule.^{16,17} A limitation of this study is that only two eyes were exposed to each substance and analysed with TEM, and of course we cannot be certain that there was no damage caused by the substance. No statistics is possible to perform with such a small sample size. Further studies are needed.

In conclusion, our results are promising regarding the use of 5-FU and thapsigargin with the Perfect Capsule. Morphologic evaluation showed that there was no damage to the posterior capsule when investigated with TEM.

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