

CELL BIOLOGICAL MECHANISMS UNDERLYING POSTERIOR CAPSULE OPACIFICATION: SEARCH FOR A THERAPY

VAN TENTEN Y.[°], SCHUITMAKER J.J.[°],
DE GROOT V.[°], WILLEKENS B.^{°°},
VRENSSEN G.F.J.M.^{°°}, TASSIGNON M.J.[°]

SUMMARY

The present paper overviews our recent studies for PCO prevention using an *in vitro* human capsular bag model and application of hyperthermia and photodynamic therapy with Bacteriochlorin A as the sensitizer as described in previous papers^{20,21}. These studies clearly showed that both treatments are successful *in vitro* and almost completely reduce the proliferation of lens epithelial cells on the posterior capsule and thus are potential candidates to eliminate the occurrence of PCO *in vivo*. Hyperthermia has a threshold temperature between 55°C and 60°C which makes this approach not very useful for *in vivo* application. Threshold conditions for PDT/BCA are much more moderate. Recent preliminary *in vivo* studies in the rabbit showed that using the *in vitro* threshold conditions, the formation of a ring of Soemmering and outgrowth of lens epithelial cells on the posterior capsule is significantly reduced. However, these conditions have an adverse affect on the corneal stroma and endothelium. Studies of PDT conditions which further reduce LEC outgrowth without affecting the corneal integrity are in progress.

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[°] Department of Ophthalmology, University Hospital Antwerp (Belgium)

^{°°} Department of Biology, Leiden University Medical Center (The Netherlands)

^{°°°} Lens and Cornea Research Unit, The Netherlands Ophthalmic Research Institute (The Netherlands)

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RÉSUMÉ

Cet article présente le résumé de nos études récentes considérant l'application de l'hyperthermie et de la thérapie photodynamique dans la prévention de la cataracte secondaire, en utilisant un modèle *in vitro* du sac capsulaire humain. Ces études montrent que ces deux traitements arrivent à réduire la prolifération des cellules épithéliales du cristallin *in vitro* en causant une destruction à peu près complète de ces cellules. Ces deux traitements sont donc des candidats potentiels pour éviter l'opacification capsulaire postérieure *in vivo*. La température nécessaire pour détruire les cellules épithéliales du cristallin est située entre 55° et 60°C; par conséquent cette approche n'est pas indiquée pour l'application chez l'homme. Par contre, le traitement photodynamique donne plus d'espoir. Des études plus récentes montrent qu'en employant ces conditions *in vivo*, la formation de l'anneau de Soemmering et la prolifération des cellules épithéliales sont fortement réduites. Malheureusement ces conditions ont un effet négatif sur le stroma et l'endothélium de la cornée. Des recherches sont en cours sur les conditions optimales dans lesquelles la prolifération des cellules épithéliales du cristallin peut être inhibée sans détruire la cornée.

KEY WORDS

Posterior capsule opacification, hyperthermia, photodynamic therapy, capsular bag model, apoptosis, histology.

MOTS-CLÉS

Opacification capsulaire postérieure, hyperthermie, thérapie photodynamique, modèle capsulaire, apoptose, histologie.

INTRODUCTION

Cataract is worldwide the most common cause of blindness. Its most successful treatment at present is extracapsular cataract extraction (ECCE) with implantation of an intraocular lens (IOL). The most common long term complication of ECCE is posterior capsule opacification (PCO) or after-cataract, with an estimated incidence of 20-50% within 5 years after surgery. It is caused by the proliferation and migration of lens epithelial cells (LECs) left in the capsular bag after extracapsular cataract extraction^{1,10}. The two types clinically distinguished are fibrosis and pearl formation. Children and young adults seem to be most susceptible to this complication⁷. The most commonly used therapeutic modality for treatment of PCO is photodisruption of the fibrotic part of the posterior capsule by a high energy Nd-YAG laser to create a clear visual axis¹. This treatment is expensive and not without medical complications, because it can give rise to increase in intraocular pressure, to retinal detachment, to damage of the intraocular lens (IOL), to cystoid macular edema or to spreading of endophthalmitis¹. In view of this, an important clinical challenge is reducing the frequency of PCO and thus the need for YAG-laser capsulotomy and its potential complications. Many attempts have been made to prevent postoperative migration and proliferation of lens epithelial cells leading to PCO, including more appropriate surgical methods and IOL designs^{2,18}, application of pharmacological and immunological agents^{8,12,13} and posterior circular continuous capsulorhexis (PCCC)^{6,19}. Despite all these efforts, none of them has actually succeeded in substantially reducing postoperative proliferation of PCO, although there is a small reduction in PCO incidence. For this reason, it is important to continue the investigations on the cellular mechanisms underlying PCO and to find new means of avoiding postoperative proliferation and migration of LECs in the capsular bag and thus the need for laser capsulotomy. The studies summarized here were designed to unravel the mechanisms of PCO in order to find a new means of PCO prevention. The present paper overviews our recent studies for PCO prevention using an *in vitro* human capsular bag model and application of hyper-

thermia and photodynamic therapy with Bacteriochlorin A

Overview of Materials and Methods

In vitro capsular bag model

For these experiments, we used the human lens capsular bag culture system, as designed by Liu et al⁹. This proved to be a reliable and consistent model, mimicking to a large extent the *in vivo* situation after cataract extraction^{4,22}. Capsular bags were prepared from donor eyes after resection of the cornea. *Ex in vivo* cataract surgery was performed, including anterior circular capsulorhexis, hydroexpression of lens fibre mass using Eagle's Minimal Essential Medium (EMEM) supplemented with 2% fetal calf serum (FCS), and aspiration of residual lens fibres. The capsular bag was then dissected together with zonules and ciliary body. The capsular bag can be cultured in EMEM medium for up to several weeks.

Hyperthermia: in vitro experiments

After heating the specimens at different temperatures (37°; 55°; 60°; 65°) in a water bath for two minutes, they were fixed either immediately or after 48 hours or 1 week of culture in EMEM supplemented with 2% FCS at 37° in a 5% CO₂ atmosphere. Following fixation, the capsular bags were dissected in parts and used for light and electron microscopy.

Photodynamic therapy with Bacteriochlorin A: in vitro experiments

Photodynamic therapy (PDT) is becoming an established cancer treatment modality. This can be attributed to the attractive basic concept of PDT; the combination of two therapeutic agents, a photosensitizing drug and light, which are relatively harmless by themselves but combined (in the presence of oxygen) ultimately cause more or less selective tumor destruction^{3,11}. It has been tested for the treatment of many cancers for instance of skin, esophagus, bladder, bronchus and lung¹⁶. In addition, treatment of preneoplastic lesions, as well as many noncancerous conditions, such as atherosclerosis, age-related macular degeneration and rheumatoid arthritis, are currently under investigation⁵. PDT involves administration of a tumor-localizing

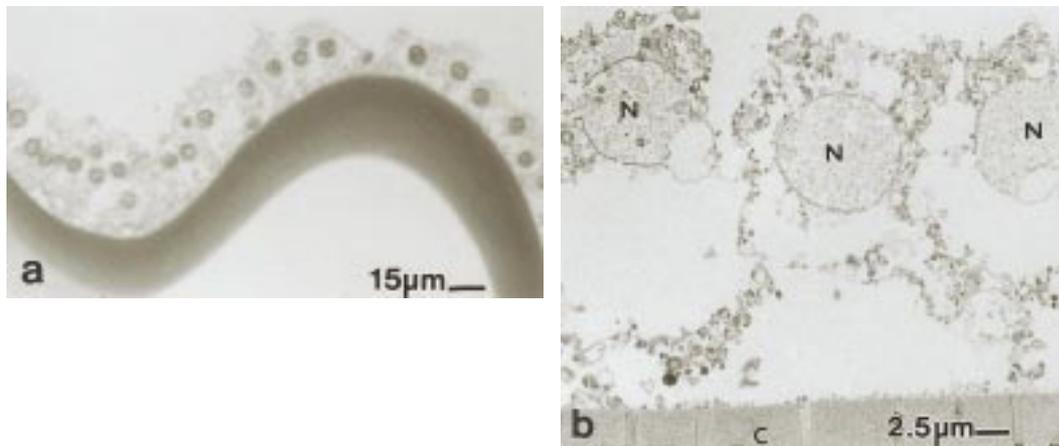


Fig 1. The effect of hyperthermia on LECs in a capsular bag model. A) Cells heated at 65°C and cultured for 2 days appear on LM as faintly stained "ghost cells" with a round nucleus. B) TEM of LECs heated at 60°C and cultured for 7 days, showing destruction of membranes of both cell organelles and nuclear envelope, few remaining cytoplasmic elements and empty nuclei (N). The capsule (C) seems to be unaffected.

photosensitizing agent, followed by activation of the agent by light with a wavelength, matching the absorption spectrum of the dye, initiating a sequence of photochemical and photobiologic processes that cause irreversible photodamage to tumor tissues^{3,11}. To date, the most commonly used sensitizer in patients is Photophrin^R, a derivative of hematoporphyrin¹¹. Although Photophrin has proven to be effective, it is not the most suitable photosensitizer for PDT, since it induces severe skin photosensitivity^{14,16}. A multitude of new sensitizers is currently under evaluation, which are likely to cause less photosensitivity. A good example of second generation photosensitizers is Bacteriochlorin A(BCA)^{14,15,17}.

BCA was obtained by saponification of Bacteriochlorophyll a and formulated in 30% of polyethylglycol, 20% of ethanol 70% and 50% of water.

After dissection, the capsular bags were incubated with BCA and subsequently illuminated using a custom-made experimental laser diode (Philips Optoelectronic Centre, Eindhoven, The Netherlands), emitting 760 nm. Different BCA concentrations, incubation times and illumination periods were tested. After a culture period of 7 days, the PDT-induced effects were evaluated using light and electron microscopy.

The threshold parameters for PDT (BCA-concentration, incubation-time and illumination-time) were determined. Untreated capsular bags, bags incubated with BCA but not illuminated and illuminated bags without BCA were used as controls.

Photodynamic therapy with Bacteriochlorin A: in vivo experiments

Albino rabbits were anesthetized with an intramuscular injection of Ketalar^R and Rompun^R. Both eyes underwent an extracapsular cataract extraction without intraocular lens implantation. One eye served as control. In the other eye, BCA was injected after removal of lens fibres from the capsular bag and incubated for 10 minutes. Subsequently, the eye was illuminated with a diode laser emitting 760nm for 15 minutes. Six weeks after surgery, the rabbits were sacrificed and the globes were enucleated. The capsular bags were dissected and examined with LM and TEM.

Histological and electromicroscopic techniques

The capsules were unfolded with the cellular layer facing upwards, rinsed in PBS and stained with haematoxylin-eosin. After dehydrating in a graded series of ethanol, they were embed-

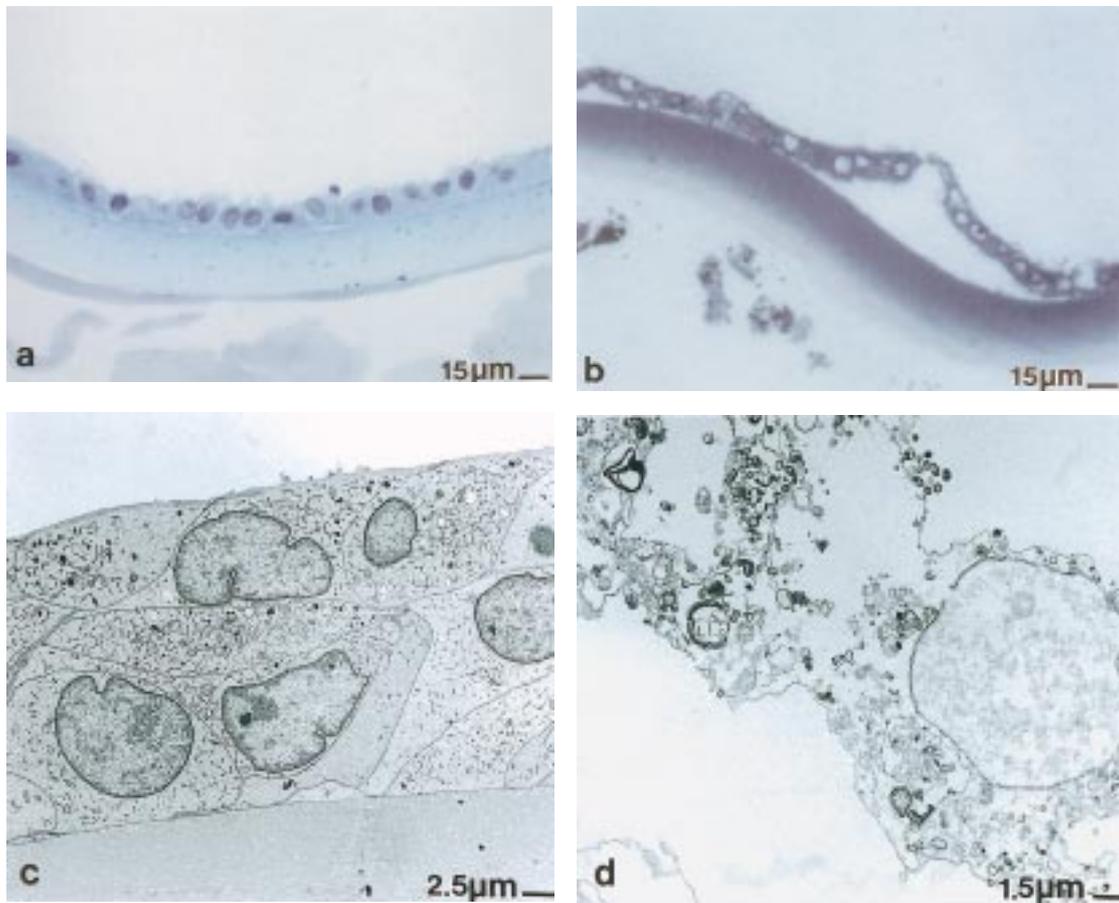


Fig 2. Capsular bags treated with PDT and BCA at optimal conditions in comparison with control capsular bags (BCA alone or illumination alone), after 7 days of culture. A) A tight monolayer of cells with dark staining nuclei is present in the control capsular bags. B) The PDT treated LECs loose adherence to the capsule and contain empty, not staining nuclei. C) TEM of control capsular bags. LECs form a tight layer and contain nuclei with patches of hetero- and euchromatin and a well differentiated cytoplasm, with many cell organelles. D) TEM of PDT treated capsular bags. Nucleus without recognisable chromatin. Cytoplasm is completely deranged.

ded in Entellan and inspected with a light microscope.

After rinsing in cacodylate buffer, the specimens were postfixed for electron microscopy in 1% osmium tetroxide supplemented with 1.5% potassium hexacyanoferrate (III) and, after dehydration in ethanol, embedded in epoxy resin. Semithin sections were stained with toluidine blue and inspected in a light microscope. Ultrathin sections were stained with uranyl acetate and lead citrate and inspected in a Philips EM 201 transmission electron microscope (Philips Industries, Eindhoven, NL).

Overview of *in vitro* results

This project was designed to unravel the mechanisms of PCO in order to find a new means of PCO prevention. In a first part, the effect of temperature on lens epithelial cells was determined *in vitro* in a human capsular bag model. The findings showed that heating up to 60°C was necessary to fully destroy the LECs left in the capsular bag after ECCE (Fig 1A-B). This really set a limit to the clinical usefulness of hyperthermia as a means of PCO prevention. It would be technically difficult to restrict this rise in temperature to the LECs and neighbouring

ocular tissue in the anterior chamber and the outer vitreous membrane would suffer from the hyperthermia²⁰.

In a following part of the project, we switched interests to the use of photodynamic therapy in the prevention of PCO.

In the *in vitro* experiments, we found that PDT with BCA is able to induce cell death in lens epithelial cells *in vitro*. The conditions which were found to be optimal for cell destruction were a BCA concentration of 10µg/ml, an incubation time of 10 minutes and an illumination time of 15 minutes. Fig 2 shows the effect of photodynamic therapy at these conditions on LECs (Fig 2B-D), compared to the untreated control (Fig 2A-C).

Using the optimal *in vitro* conditions, *in vivo* studies on rabbit eyes were performed. The preliminary results showed that the LECs showed an extensive ring of Soemmering in the control capsular bags. In the treated capsular bags the formation of a ring of Soemmering and outgrowth of lens epithelial cells on the posterior capsule is significantly reduced. However, these conditions have an adverse affect on the corneal stroma and endothelium. Studies on PDT conditions which further reduce LEC outgrowth without affecting the corneal integrity are in progress.

CONCLUSION

PCO is a still existing problem after cataract surgery. PDT with BCA is a promising new approach in the prevention of PCO. Further *in vivo* experiments have to be carried out to optimise BCA concentration, incubation time and illumination time.

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- Corresponding author*
 van Tenten Yasmine
 University Hospital of Antwerp
 Wilrijkstraat 10
 2650 Edegem
 tel +32 3 821 33 79
 fax +32 3 825 19 26
 e-mail yvantenten@hotmail.com