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LABORATORY STUDY

Irrigation with thapsigargin and various concentrations of 5-fluorouracil in a sealed-capsule irrigation device in young rabbit eyes to prevent after-cataract

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Abstract

Aim **To investigate the effect on after-cataract and synechiae formation after clear lens extraction and irrigation with different substances in a sealed-capsule irrigation device.**

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

Methods **Clear lens extraction was performed in one eye of 42 4-week-old rabbits. The sealed-capsule irrigation device was applied and the sealed system was irrigated for 2 min with 20 ml of one of four substances: balanced salt solution (BSS), thapsigargin, 5-fluorouracil (5-FU) 50 mg/ml, or 5-FU 25 mg/ml. The substance then was washed out for 10 s with BSS. The eyes were left aphakic. Formation of after-cataract and synechiae was evaluated during two clinical examinations 3.5 and 5.5 weeks postoperatively, by photographs 5 weeks postoperatively, and by histologic evaluation of haematoxylin–eosin-stained slides after the 6-week end point postoperatively. After-cataract and synechiae were graded on a scale from 0 to 4. Kruskal–Wallis analysis of variance with multiple comparisons was used for statistical analyses.**

Results **5-FU 50 mg/ml prevented after-cataract and synechiae formation best when compared with all other substances at all evaluations. 5-FU 25 mg/ml was not as effective, and thapsigargin was ineffective in this animal model.**

Conclusion **5-FU 50 mg/ml used in the sealed-capsule irrigation device satisfactorily prevents after-cataract and synechiae. Thapsigargin was ineffective in this animal model.**

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Keywords: sealed-capsule irrigation; perfect capsule; fluorouracil; thapsigargin; paediatric cataract surgery; posterior capsule opacification

Introduction

Posterior capsule opacification (PCO) is a common complication after cataract surgery.[1] In children, the younger the child, the more extensive is the after-cataract that develops[2] and a posterior capsulorhexis[3] and anterior vitrectomy also can help reducing the amount.[4–7] However, in the smallest children, an anterior vitrectomy is not always sufficient, but visual axis opacification (VAO) may develop and require a secondary procedure to remove the lens epithelial cells (LECs) on the back of the optic and in the residual vitreous.[8,9] A new sealed-capsule irrigation device, that is, Perfect Capsule (Milvella, Ltd, Epping, Australia),[10] might be helpful to prevent VAO in children. The capsule is left intact, the Perfect Capsule is sealed to the capsule with a vacuum, and the system can be flushed with a substance to

remove all the LECs. Initially, it was believed that distilled deionized water would remove the LECs through osmotic lysis. However, a recent study of human adults found that irrigation with distilled deionized water had no effect on PCO.[11]

We previously found that 5-fluorouracil (5-FU) 50 mg/ml effectively killed LECs in a rabbit model.[12] We also showed that it is safe to use 5-FU with the device.[13] However, since 5-FU is an antimetabolite and potentially toxic, it is crucial to identify the lowest concentration at which 5-FU is effective. We also evaluated thapsigargin, a hydrophobic inhibitor of endoplasmic reticulum (Ca^{2+})-ATPase, which has proved effective in *in vitro* studies of human lens capsules.[14,15]

This study was conducted to compare balanced salt solution (BSS), 5-FU 50 mg/ml, 5-FU 25 mg/ml, and thapsigargin in a sealed-capsule irrigation device to determine the substance that is most effective in preventing after-cataract formation and postoperative inflammation.

Materials and methods

Animals

Forty-two New Zealand white rabbits, 4 weeks old (average weight, 302 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

Before surgery, the pupils were fully dilated with a mixture of cyclopentolate 0.75% and phenylephrine 2.5%. The surgeries were performed by one experienced anterior segment surgeon (CZ). The animals were deeply anaesthetized with a mixture of ketamine hydrochloride and Xylocaine chloride. Topical anaesthesia (tetracaine chloride eye drops) was applied to each eye. A 2.8-mm corneal incision was created with a disposable bevel-up 45-degree slit knife. Healon GV (Advanced Medical Optics) was injected into the anterior chamber followed by creation of a tear-resistant capsulorhexis of about 3 mm. The capsulorhexis had to be less than 4–4.5 mm to allow the Perfect Capsule to fit and seal tightly because the inner diameter is 5 mm, and the rhexis has to be fully covered by the device to obtain a vacuum and remained sealed throughout the procedure. Irrigation/aspiration of the lens material was carried out using BSS with heparin 10 000 IU/l.

The Perfect Capsule was folded with forceps and inserted through the small incision, pushed down onto the anterior capsule, and a vacuum syringe was pulled back and locked to create the vacuum, holding the device in place during irrigation. The sealed system with the capsule then was irrigated with one of four substances for 2 min, followed by 10 s of BSS flush to wash out the residual substance.

After irrigation was completed, the vacuum was released and the Perfect Capsule was removed. The corneal incision was closed using a continuous 10-0 nylon suture. One milligram of cefuroxime was injected intracamerally. Starting from the day after surgery, the rabbits received dexamethasone eye drops 1 mg/ml four times daily in the operated eye, the dose of which was later tapered to twice daily during the last 2 weeks.

Study design

Ten eyes in group 1 were irrigated with BSS, 11 eyes in group 2 were irrigated with thapsigargin 300 μM (Sigma), 10 eyes in group 3 were irrigated with 5-FU 50 mg/ml, and 11 eyes in group 4 were irrigated with 5-FU 25 mg/ml. During the 2-min irrigation period, about 20 ml of each substance was washed through the system.

To evaluate the effects of irrigation with different 5-FU concentrations and thapsigargin on after-cataract, we evaluated after-cataract and synechiae formation during two clinical examinations 3.5 and 5.5 weeks postoperatively, retroillumination photographs that were obtained 5 weeks postoperatively, and histologic evaluation. In these eyes, it was not possible to perform any digital evaluation of PCO, as the posterior synechia often was extensive, and no red reflex then was obtained. We therefore had to subjectively grade the after-cataract. Measurements and examinations were performed through fully dilated pupils (with a mixture of cyclopentolate 0.75% and phenylephrine 2.5%) and in a masked manner, that is, the examiner did not know which solution had been used in the eye. After-cataract and synechiae were graded on a scale from 0 to 4, where 0 indicated no after-cataract or a totally round large pupil, 1 indicated minimal after-cataract or some peripheral posterior synechiae, 2 indicated medium after-cataract or a pupil not totally attached by synechiae but about half of the pupil. Three indicated moderate after-cataract or a pupil almost covered by posterior synechiae and 4 indicated extensive after-cataract and a pupil totally covered by posterior synechiae.

All animals were killed 6 weeks after surgery. All but two eyes in each group were extracted and fixated in formalin for histologic evaluation. Two eyes in each group were fixed for later transmission electron microscopy evaluation of the posterior capsule not described here. The eyes fixated in formalin were sectioned, mounted, and stained with haematoxylin and

eosin and analysed for after-cataract; however, it was not possible to evaluate posterior synechiae histologically.

Statistical analysis

Kruskal–Wallis analysis of variance with multiple comparisons was used to calculate the difference between the groups regarding the formation of after-cataract and synechiae.

Results

The detailed results of after-cataract and synechiae formation are shown in Tables 1 and 2. Thapsigargin (Figure 1) was ineffective in preventing LEC proliferation in young rabbits, and the substance was not associated with less after-cataract ($P > 0.05$ in all four evaluations) or synechiae ($P > 0.05$ in all three evaluations) than BSS (Figure 2). Irrigation with 5-FU 50 mg/ml (Figure 3) resulted in less after-cataract ($P < 0.01$ in all four evaluations) and synechiae ($P < 0.001$ in all three evaluations) than BSS. Irrigation with 5-FU 25 mg/ml (Figure 4) resulted in less after-cataract than BSS at the first clinical examination and at the photographic and histologic evaluations ($P < 0.05$) but not at the second clinical examination ($P > 0.05$). 5-FU 25 mg/ml also was associated with less synechiae than BSS in all three evaluations ($P < 0.05$).

Discussion

Our data showed that 5-FU 50 mg/ml seems to be the most-effective substance of all the substances tested for preventing after-cataract and synechiae formation in this animal model. 5-FU 50 mg/ml was the most-effective agent in all four evaluations. In the current study, we wanted to determine if it was possible to lower the 5-FU concentration; however, at least in the young rabbit, a concentration of 50 mg/ml is needed.

In a recent study, 5-FU at a low concentration of 12.5 mg/ml was administered during hydrodissection in the rabbit eye, and no inhibitory effect of LEC proliferation was seen.[16] In another study,[17] clear lens extraction was performed in rabbit eyes followed by injection of a drug mixed with an ophthalmic viscosurgical device (OVD) in the capsular bag. In two rabbits, the investigators injected 5-FU 33 mg/ml mixed in the OVD in the bag, left the OVD in the capsular bag for 3 min, then rinsed it out. The concentration, however, was probably lower when the drug is mixed with OVD, and there was no irrigation, but merely contact with the mixed OVD for 3 min. In this study, 5-FU did not effectively prevent PCO. A high concentration of 5-FU seems to achieve the inhibitory effect *in vivo* in the rabbit eye. Also, our study differs from the above-mentioned studies, in that, as the capsule was empty and exposed only to pure 5-FU when using the Perfect Capsule, and the LECs should therefore be more sensitive. Even so, in our model, a concentration of 50 mg/ml was needed.

Thapsigargin was ineffective in preventing LEC proliferation and migration in this young rabbit model; however, it has earlier reported to be effective in killing human LECs.[14,15] Coating polymethylmethacrylate lenses with thapsigargin effectively prevented LEC proliferation in an *in vitro* study of human capsular bags.[14] In an extensive study of rabbit and human

Table 1 Comparison of after-cataract formation at four different evaluations

After-cataract	P-*values compared to BSS*		
	5-FU 50 mg/ml	*5-FU 25 mg/ml*	*Thapsigargin 300* μM
Clinical examination 1	0.00015	0.0039	0.76
Clinical examination 2	0.0073	0.15	1.0
Photographic evaluation	0.00072	0.0062	1.0
Histologic evaluation	0.0022	0.037	0.19

Table 2 Comparison of synechiae formation at three different evaluations

Synechiae	P-*values compared to BSS*		
	5-FU 50 mg/ml	*5-FU 25 mg/ml*	*Thapsigargin 300* μM
Clinical examination 1	0.00017	0.0072	1.0
Clinical examination 2	0.00028	0.012	1.0
Photographic evaluation	0.000006	0.00044	0.80

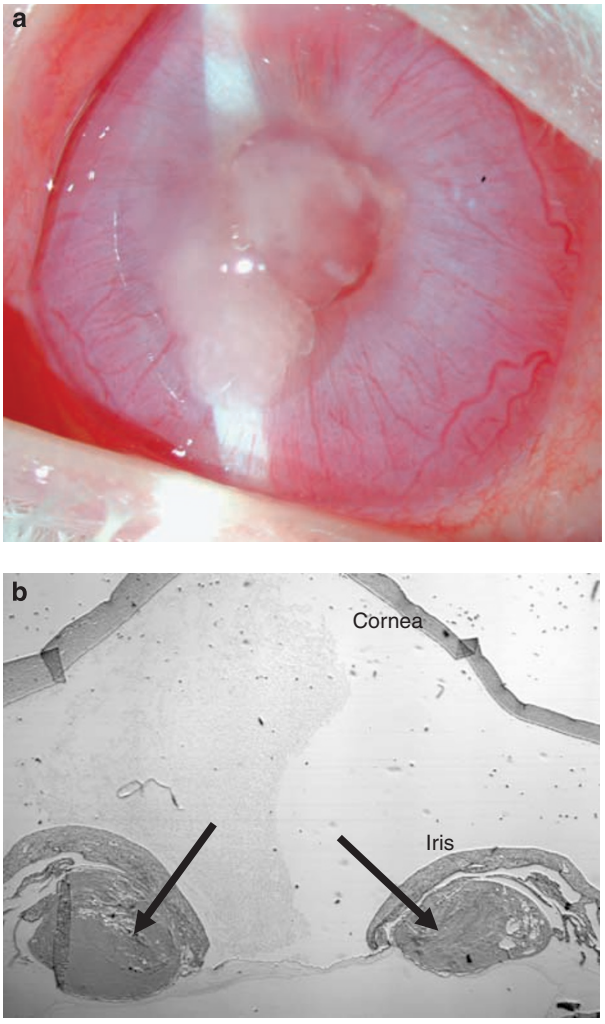

Figure 1 (a) Slit-lamp retroillumination photograph of an eye irrigated with thapsigargin. After-cataract is seen also in the anterior chamber, and the pupil is small with synechiae attached. (b) Histologic slide (a $\times 4$ magnification objective was used) shows an eye irrigated with thapsigargin. The arrows indicate Sommering's ring.

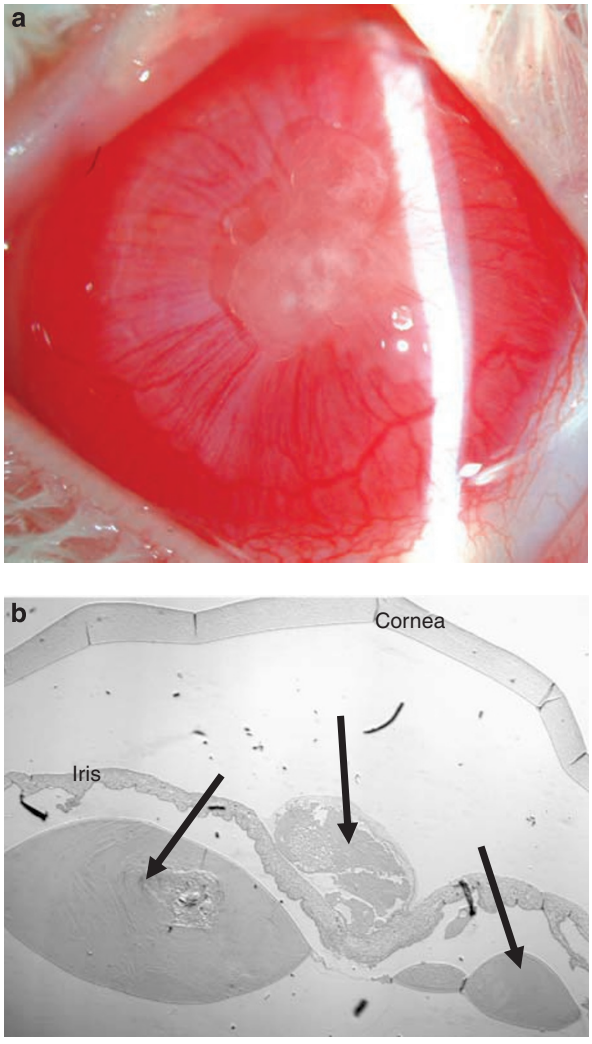

Figure 2 (a) Slit-lamp retroillumination photograph of an eye irrigated with BSS. After-cataract is seen also in the anterior chamber, and the pupil is small with synechiae attached. (b) Histologic slide (a $\times 4$ magnification objective was used) shows an eye irrigated with BSS. The arrows indicate Sommering's ring and after-cataract in the anterior chamber.

capsular bags *in vitro*, Duncan *et al*[15] found that LEC growth in rabbit capsular bags exposed to thapsigargin was not inhibited by the substance; however, in bags exposed to 5-FU 25 mg/ml, LEC growth was totally inhibited. When performing the same experiment in human donor capsular bags, thapsigargin totally inhibited cell proliferation, and 5-FU 25 mg/ml almost totally inhibited proliferation, although some live cells were seen after 28 days in the outer region of the human capsular bags. Duncan *et al*[14] also performed cell cultures of human LECs (FHL124 cells), in which the cells were exposed to different substances for 2 min. In cell culture, thapsigargin and 5-FU 25 mg/ml effectively killed the cells. The explanation for why thapsigargin is more effective in human LECs than in rabbit LECs is unknown; however, Duncan *et al*[14] proposed that the rabbit model often uses young animals whereas the human donor eyes most often are older. The rabbit cells are, therefore, more sensitive to stress than the human cells. In addition, rabbit cells divide faster than aged human cells and 5-FU mainly targets rapidly dividing cells. We focused primarily on the use of the Perfect Capsule in children's eyes. They also have very rapidly dividing cells, which is why they develop more extensive after-cataract compared to adults. Therefore, 5-FU might be a good substance to use in paediatric eyes. Thapsigargin might be more promising in adult eyes, in which the use of the Perfect Capsule is mostly indicated in cases in which a posterior capsulotomy cannot be performed, such as in

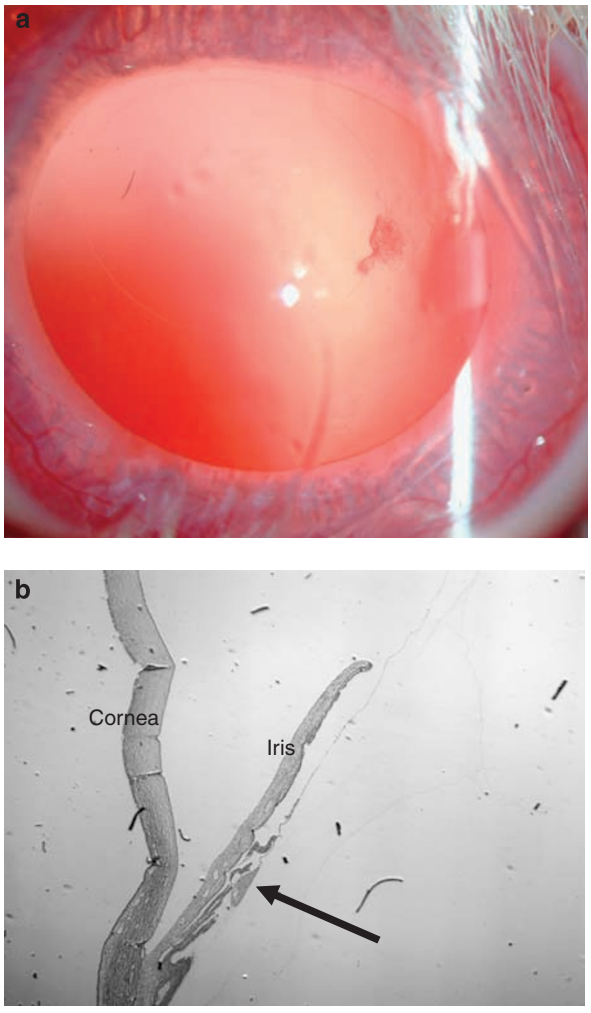

Figure 3 (a) Slit-lamp retroillumination photograph of an eye irrigated with 5-FU 50 mg/ml. Almost no after-cataract is seen and the pupil does not have synechiae but is round and well dilated. (b) Histologic slide (a ×4 magnification objective was used) shows an eye irrigated with 5-FU 50 mg/ml. The arrow indicates minute after-cataract at the equator of the capsular bag.

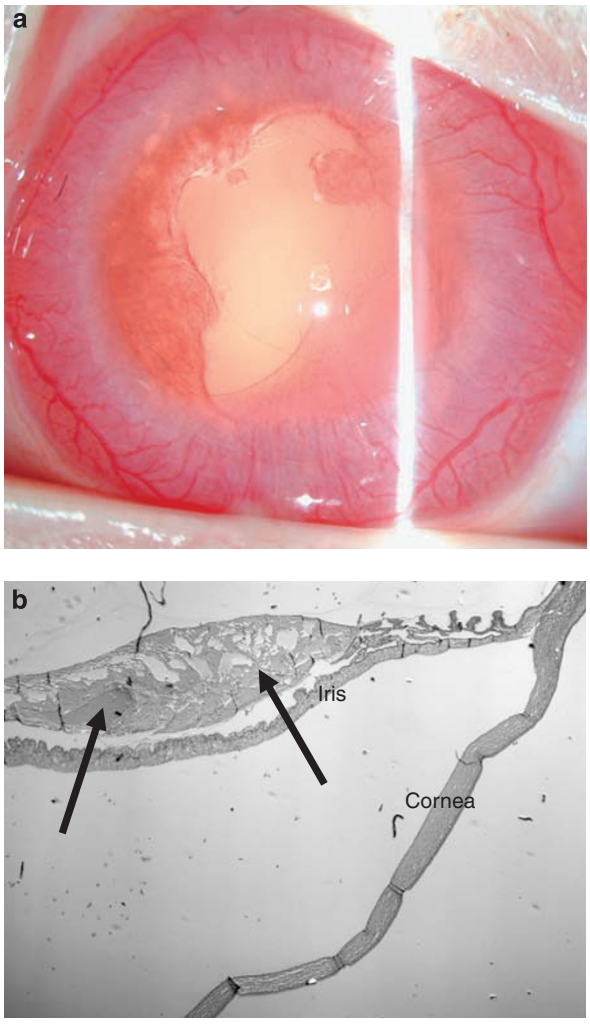

Figure 4 (a) Slit-lamp retroillumination photograph of an eye irrigated with 5-FU 25 mg/ml. Some after-cataract is seen in the periphery and the pupil has some synechiae. (b) Histologic slide (a ×4 magnification objective was used) shows an eye irrigated with 5-FU 25 mg/ml. The arrows indicate after-cataract centrally.

patients with accommodative intraocular lenses of different material.

When using the Perfect Capsule, the system is supposed to be sealed all the time; however, the vacuum can be incomplete and hypothetically a substance can escape into the anterior chamber. We showed previously that 5-FU 50 mg/ml is non-toxic to the adjacent intraocular structures.[13] When 5-FU was injected in the bag after clear lens extraction without using the Perfect Capsule, other ocular tissues such as the corneal endothelial cells, trabecular meshwork, and retina were intact. Even when 5-FU was left in the capsular bag after irrigation and not washed out with BSS, the other tissues were unaffected. 5-FU is often used in glaucoma surgery and also in paediatric cases.[18,19] 5-FU also has been used intravitreally to manage retinal detachments.[20,21] Furthermore, it has been shown that intraocular use of 5-FU is safer for the corneal endothelium than mitomycin C.[22]

In the current study, irrigation with the substances lasted for 2 min. In our earlier studies, irrigation was continued for 5 min. As the rabbit model is not fully representative of the human eye, studies in humans are needed to determine the length of the irrigation time that prevents development of after-cataract in children.

One limitation of the current study is that the surgeon is not masked to the solution used. This can of course create bias. Another limitation of the study is that the grading of the after-cataract is subjective, but in our model, this is difficult to get around. The rabbits were kept aphakic in our study, although implantation of

intraocular lenses has become more common in paediatric cataract surgery also. If an intraocular lens is implanted, less after-cataract and synechiae develop[23] and it is then more difficult to see the difference between the groups, which is why we chose to leave the eyes aphakic.

In conclusion, the current study showed that in this young rabbit model, a concentration of 50 mg/ml is needed for 5-FU to prevent formation of after-cataract and synechiae. Thapsigargin was ineffective in this model.

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