In Vivo Inhibition of Lens Regrowth by Fibroblast Growth Factor 2–Saporin

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Purpose. To investigate the ability of fibroblast growth factor (FGF) 2–saporin to prevent lens regrowth in the rabbit.

Methods. Chemically conjugated and genetically fused FGF2–saporin (made in Escherichia coli) were used. Extracapsular extraction of the lens was performed on the rabbit, and the cytotoxin either was injected directly into the capsule bag or was administered by FGF2–saporin-coated, heparin surface-modified (HSM) polymethylmethacrylate intraocular lenses. The potential of the conjugate was checked by slit lamp evaluation of capsular opacification and by measuring crystallin synthesis. Toxin diffusion and sites of toxin binding were assessed by immunohistochemistry. Possible toxicity was determined by histologic analysis of ocular tissues.

Results. FGF2–saporin effectively inhibited lens regrowth when it was injected directly into the capsular bag. However, high concentration of the toxin induced transient corneal edema and loss of pigment in the iris. Intraocular lenses coated with FGF2–saporin reduced lens regrowth and crystallin synthesis without any detectable clinical side effect. After implantation, FGF2–saporin was shown to have bound to the capsules and, to a lesser extent, to the iris; no histologic damage was found on ocular tissues as a result of implantation of drug-loaded HSM intraocular lenses.


The development of secondary cataracts caused by the growth of lens epithelial cells can compromise the visual benefit of surgery. Visual acuity can be restored by Nd:YAG laser posterior capsulotomy,1,2 but opening the posterior lens capsule can lead to severe complications, including macular edema, retinal detachment, or increased intraocular pressure.3,4 Several antimitotic drugs have been tried to inhibit capsule opacification, but without great success, and these drugs are not widely accepted for clinical use.5–10

A previous study11 suggested that the plant antimitotic, saporin, when covalently linked to basic fibroblast growth factor (FGF2) to form FGF2–saporin, might be suitable for preventing lens cell proliferation over the posterior lens capsules. We showed that bovine lens epithelial cells possess receptors for FGF2 and respond to the growth factor. Basic fibroblast growth factor also binds strongly to heparan sulfate on the surface of the lens capsule.12 These properties suggest that FGF2–saporin could be an efficient way of preventing capsule opacification after extracapsular extraction of the lens. The in vitro cytotoxicity of FGF2–saporin for bovine epithelial lens cells and the in vitro inhibition of bovine epithelial lens cell proliferation on capsules treated with FGF2–saporin support this possibility.15

The inherent affinity of FGF2 for heparin14 was used to deposit FGF2–saporin on heparin surface-modified (HSM) polymethylmethacrylate (PMMA) intraocular lenses (IOLs). These IOLs coated with FGF2–saporin (either FGF2–SAP or rFGF2–SAP) might be used to deliver the mitotoxin to the posterior lens capsule when the IOL is inserted into the pa-
In Vivo Inhibition of Lens Regrowth by FGF2-Saporin 2435

Animals

In vitro experiments on IOLs confirmed that coating HSM IOLs with FGF2–SAP or rFGF2–SAP inhibits the proliferation of bovine epithelial lens cells on the lens surface, suggesting that active FGF2-saporin binds to heparan sulfate sites of the lens surface.

The current in vivo study examines the capacity of FGF2–saporin to prevent lens regeneration in rabbits after extracapsular lens extraction (ECLE). The FGF2-saporin (either FGF2–SAP or rFGF2–SAP) cytotoxin either was injected into the capsular bag of the eye, without IOL implantation and after simple ECLE, or it was bound to HSM PMMA IOLs implanted into the capsule bag. The potential of the conjugate was checked by evaluating capsule opacification and measuring crystallin synthesis independently in these two treatment groups. The sites that bound toxin and toxin diffusion were assessed by immunohistochemistry. Possible toxic effects of the drug were determined by histologic analyses of ocular tissues.

MATERIALS AND METHODS

Animals

The rabbits used in this study were cared for and treated in accordance with the ARVO Statement for the Use of Animals in Ophthalmic and Vision Research. A total of 112 eyes of Fauves de Bourgogne female rabbits (2.5 to 3 kg, 10 to 12 weeks of age) were treated surgically. Only one eye was treated in each animal. Pigmented rabbits were used to mimic human physiological conditions. Treatment schedules and mortality are detailed in Table 1.

Treatments

Two main types of treatment were administered after ECLE. In one, FGF2–saporin (either FGF2–SAP or rFGF2–SAP) was injected directly into the capsular bag. To reduce diffusion of the drug, the injected FGF2–SAP was mixed with sodium hyaluronate in one subgroup of rabbits. The inflammation and increased intraocular pressure produced by the hyaluronate prevented evaluation of this subgroup. The second treatment group was fitted with PMMA–IOL, and the effects of coating these lenses with FGF2–saporin (either FGF2–SAP or rFGF2–SAP) were assessed. Control eyes were treated by injecting saporin intracameraly without surgery or by injecting saporin into the capsular bag after ECLE. Other controls were injected with FGF2 or heat-treated FGF2–SAP after ECLE.

Some rabbits were killed soon after surgery to determine the distribution and loss of FGF2–SAP and its early toxicity. Other rabbits were kept for 2 to 3 months to evaluate the clinical effects of the cytotoxin on lens regrowth.

Surgical Procedure: Extracapsular Extraction of Lens

Lenses were extracted using a slightly modified version of the procedure described by Jay and Green. All surgery was performed by the same surgeon to minimize variability in technique. Pupils were dilated with topical 10% neosynephrine (Chibret, Clermont–Ferrand, France) and tropicamide (Mydriaticum; Merck Sharp & Dohme–Chibret) administered every 10 minutes starting 1 hour before surgery. The rabbit was sedated with 2 mg/kg nidazolam (Hynovel; Roche, Neuilly sur Seine, France) administered intramuscularly, and it was anesthetized with 60 mg/kg ketamine administered intramuscularly (Ketalar, Parke–Davis, Panpharma, Fougeres, France). The rabbit was then placed under an operating microscope in a stereotaxic head device. The eye was cleaned with 0.4% iodine solution (Betadine; Sarget, Asta, Mérignac, France) and gentamycin drops (Gentalline; Plough–Schering, Levallois–Perret, France), and the eyelashes were trimmed. Sterile surgical procedures were used. The ocular globe was exposed by retracting the ocular muscles with a 2-0 silk suture. A large 10° anterior corneal incision was made with a 45° blade, beginning at the 12-o’clock position. Capsulotomy was performed with a cystotome by bending the tip of a 25-gauge needle connected to a Healon syringe. A linear 5-mm incision was made for simple extracapsular extraction. Care was taken to cut only the capsule and not to enter the nucleus. To ensure that the IOL was in the capsular bag, capsulorrhexis of approximately 5 to 6 mm in diameter was made when a posterior chamber IOL was implanted in the capsular bag. Anterior chamber depth was maintained during capsulotomy by the injection of Healonid (Pharmacia, St. Quentin, Yuelines, France). The corneal incision was extended to 100° with corneal scissors, and the nucleus was removed in one piece by pressure. All Healonid was removed by washing the anterior chamber with balanced salt solution (BSS; Alcon, Rueil–Malmaison, France), and the incision was closed with a running 9-0 nylon suture. Finally, 300 μl of cytotoxin (0.1 μM or 1 μM FGF2–SAP or rFGF2–SAP in BSS) was injected into the capsule bag with a cannula. Six rabbits were treated by injecting 240 μl of a 1 μM solution of FGF2–saporin in Healonid into the capsule bag. Healonid (sodium hyaluronate) was used to open the capsule bag before insertion of the HSM PMMA IOL. 720c Pharmacia IOLs, one-piece PMMA biconvex, 13.5 mm, 10° angulated haptics, were used. The hyaluronate was always washed out with BSS, and 1% pilocarpine (Chibret) drops were instilled to obtain myosis after implantation. The incision was closed with a running 9-0 nylon suture, muscle tractions were...
**TABLE 1. Summary of Treatment Schedules and Mortality**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Number of Eyes</th>
<th>Day Killed</th>
<th>Fate</th>
</tr>
</thead>
<tbody>
<tr>
<td>ECLE without IOL</td>
<td>12</td>
<td>1, 6 hours</td>
<td>immuno</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 (dead), 1 day</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 1 day</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 5 days</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 15 days</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>7, 25 to 32 days</td>
<td>slit-lamp, proteins</td>
</tr>
<tr>
<td>ECLE + FGF2-SAP injection</td>
<td>17</td>
<td>2 (1 dead), 6 hours</td>
<td>immuno</td>
</tr>
<tr>
<td>(1 corneal abscess)</td>
<td></td>
<td>2, 1 day</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 (1 dead), 5 days</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 15 days</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>8, 25 to 32 days</td>
<td>immuno, slit-lamp, proteins, histo</td>
</tr>
<tr>
<td>ECLE + rFGF2-SAP injection</td>
<td>6</td>
<td>1 dead per op</td>
<td>excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 corneal abscess</td>
<td>excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 5 days</td>
<td>histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1, 21 days</td>
<td>histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>4 (2 dead), 65 to 70 days</td>
<td>histo, proteins evaluation</td>
</tr>
<tr>
<td>ECLE + Healonid (+/− FGF2-SAP)</td>
<td>6</td>
<td>6, 65 to 70 days</td>
<td>slit-lamp, no opacification</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 endophthalma</td>
<td>slit-lamp, protein</td>
</tr>
<tr>
<td>ECLE + IOL</td>
<td>24</td>
<td>9, 2 months</td>
<td>slit-lamp, protein</td>
</tr>
<tr>
<td>(1 endophthalma)</td>
<td></td>
<td>9, 3 months</td>
<td>slit-lamp, protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2, 1 day</td>
<td>histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 endophthalma</td>
<td>excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3 IOL luxation</td>
<td>excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 dead, 2 months</td>
<td>histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 dead, 40 days</td>
<td>histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2 IOL luxation</td>
<td>excluded</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2, 1 day</td>
<td>immuno, histo</td>
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<td></td>
<td>2, 5 days</td>
<td>immuno, histo</td>
</tr>
<tr>
<td></td>
<td></td>
<td>9, 2 months</td>
<td>slit-lamp, protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6, 3 months</td>
<td>slit-lamp, protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>6, 2 months</td>
<td>slit-lamp, protein</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 dead, 10 days</td>
<td>excluded</td>
</tr>
<tr>
<td>ECLE + rFGF2-SAP coated IOL</td>
<td>7</td>
<td>10</td>
<td>2, 2 months</td>
</tr>
<tr>
<td>SAP-3 ECLE + SAP control-2 SAP</td>
<td></td>
<td>1 (dead) per op</td>
<td>histo</td>
</tr>
<tr>
<td>injection-5 ECLE + SAP coated</td>
<td></td>
<td>2, 2 months</td>
<td>slit-lamp, histology</td>
</tr>
<tr>
<td>IOL</td>
<td></td>
<td>2, 1 day</td>
<td>slit-lamp, histology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>3, 2 months</td>
<td>slit-lamp, histology</td>
</tr>
<tr>
<td>ECLE + FGF2 injection</td>
<td>3</td>
<td>2, 2 months</td>
<td>slit-lamp, histology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 dead per op</td>
<td>histo</td>
</tr>
<tr>
<td>ECLE + heated FGF2-SAP injection</td>
<td>3</td>
<td>2, 2 months</td>
<td>slit-lamp, histology</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1 dead, 23 days</td>
<td>excluded</td>
</tr>
</tbody>
</table>

ECLE = extracapsular lens extraction; IOL = intraocular lens; FGF2 = fibroblast growth factor 2; rFGF2 = recombinant fibroblast growth factor 2; SAP = saporin; histo = histology; immuno = immunolocalization; per op = peroperatory; proteins = protein assay.

released, and dexamethasone–neomycin ointment (Chibrocadron; Chibret) was applied to the surface of the eye. Tropicamide and dexamethasone–neomycin drops were instilled 3 times a day each day for 1 month after surgery.

**Evaluation of Capsular Clouding**

The anterior segment was examined by slit lamp biomicroscopy and photographed 6, 15, 25 to 30, 45, 60 to 65 days, 3 months, and 4 months after surgery. Lens regrowth was evaluated independently by two ophthalmologists by slit lamp examination in a blind design using the scale: 0 = clear capsule; 0.5 = slight clouding in some fields; 1 = clouding 1+; 2 = clouding 2+; 3 = clouding 3+; 4 = clouding 3+ and/or de novo crystallins in the pupillary area.

**Evaluation of Residual Cortex After Lens Extraction**

Ten rabbits were killed 6 to 24 hours after lens extraction, and the capsular bags were examined. Back-lit pictures of the capsule bag with the IOL inside were taken of some rabbits during excision. The other rabbits were used for microscopic analysis of the inside.
In Vivo Inhibition of Lens Regrowth by FGF2–Saporin

**TABLE 2. Clinical Findings 1 Month After Extracapsular Lens Extraction**

<table>
<thead>
<tr>
<th>Rabbit Number</th>
<th>Day After Surgery</th>
<th>Lens Regeneration</th>
<th>Iris Loss of Pigment</th>
<th>De Novo Protein Synthesis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>32</td>
<td>1</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>10</td>
<td>25</td>
<td>0.5</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>11</td>
<td>30</td>
<td>4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>14</td>
<td>30</td>
<td>4</td>
<td>–</td>
<td>+</td>
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<tr>
<td>25</td>
<td>30</td>
<td>4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>27</td>
<td>45</td>
<td>4</td>
<td>–</td>
<td>+</td>
</tr>
<tr>
<td>Treated</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>30</td>
<td>0.5</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>25</td>
<td>0</td>
<td>+, concentrated</td>
<td>–</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>0.5, pigments</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>12</td>
<td>30</td>
<td>2, pigments</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>13</td>
<td>30</td>
<td>0.5, pigments</td>
<td>+/-</td>
<td>–</td>
</tr>
<tr>
<td>22</td>
<td>45</td>
<td>0</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>23</td>
<td>33</td>
<td>1, pigments</td>
<td>++</td>
<td>–</td>
</tr>
<tr>
<td>24</td>
<td>45</td>
<td>0.5, pigments</td>
<td>++</td>
<td>–</td>
</tr>
</tbody>
</table>

Fifteen rabbits (seven control and eight treated) had a slit lamp examination with pictures taken at days 4, 8, 15, and 20 and just before they were killed. Capsule clouding was evaluated independently by two ophthalmologists. Unpaired *t* test, *P* = 0.005, considered very significant.

Control sections were incubated with PBS and normal calf serum. Sections were examined and photographed under ultraviolet light.

**Immunolocalization of FGF2–Saporin With Anti-Saporin Antibodies**

FGF2–SAP was detected with a goat anti-saporin antibody (Prizm Pharmaceuticals, San Diego, CA). The sites of FGF2–SAP binding and the duration of cytotoxin remaining in the eye were determined on rabbits killed 6 hours and 1, 5, and 15 days after surgery. Sections of the capsular bag alone were made at 1, 5, and 15 days after surgery. Serial sections (10 to 15 μm) of tissue specimens were cut in a cryostat and embedded in O.C.T. (Tissue-Tek, Miles, Elkhart, IN). The sections were air-dried for 30 minutes and rinsed 3 times (10 minutes each) with phosphate-buffered saline (PBS). The membranes were made permeable by incubating the sections for 5 minutes 0.2% Triton X-100 in PBS. Excess fluid was removed, and the sections were rinsed in PBS. Nonspecific sites were blocked by incubation with 5% normal rabbit serum, 1% bovine serum albumin in PBS for 30 minutes. They were then blotted and incubated with goat anti-saporin serum (diluted 1:100 in PBS, 0.05% Tween-80, 1% skim milk powder) for 1 hour in a humidified box. The sections were rinsed 4 times (5 minutes) with PBS–Tween–skim milk and incubated with fluorescein isothiocyanate-conjugated anti-goat immunoglobulin (PRIZM, diluted 1/100 in PBS–TWEEN) for 1 hour. Finally, the sections were washed 4 times (5 minutes) with PBS–Tween–skim milk and 4 times (10 minutes) in PBS and mounted in PBS–glycerol (1:1).

**Macroscopic and Microscopic Analysis of Tissue Specimens**

The globe was cut equatorially and photographed under back illumination to show the capsular bag in situ. The capsular bag was then removed, with or without the IOL inside, by cutting the zonule and was frozen for protein assay or fixed in 4% paraformaldehyde and stained with hemalum–eosin. Central areas of capsular bags were also photographed. The cornea, the ciliary body, the iris, and the posterior segment of the eye were fixed and embedded in paraffin for histologic examination.

**Electrophoresis of the De Novo Synthesized Proteins**

Newly synthesized soluble proteins in four lenses were analyzed by polyacrylamide gel electrophoresis. Three rabbits that underwent an ECLE and three that underwent ECLE plus FGF2–SAP injection were killed 1 month after surgery. Capsular bags of these six animals were analyzed for newly synthesized protein. The lens and the capsular bags were freeze-thawed several times to break cell membranes, and soluble proteins were extracted with double-distilled water, collected by centrifugation (5000g for 30 minutes) and lyophilized. The lyophilisates were dissolved in water and placed in Laemmli sample buffer, heated for 2 minutes at 100°C, and electrophoresed on 10% acrylamide gels.
FIGURE 1. Slit lamp photographs after ECLE with rFGF2-SAP injected into the capsular bag. (A) Eight days after surgery. Important corneal edema. (B) Three weeks after surgery, on the same eye, the cornea is clear but peripheral neo-vessels are observed.

slab gels together with molecular weight markers. Gels were stained with Coomassie blue R-250, and the band patterns were examined.

Dosage of Soluble Proteins From the Capsular Bag
Capsular bags were homogenized in double-distilled water, freeze-thawed several times to break cell membranes, and centrifuged at 5000g for 25 minutes, and the supernatant was lyophilized. They lyophilized protein was taken up in distilled water and assayed by the Bradford method (Bio-Rad protein assay; GMBH, Munich, Germany) with bovine serum albumin as standard.

FIGURE 2. Slit lamp photographs showing iris loss of pigment after the cytotoxin was injected in the capsule bag after ECLE. (A) Three weeks after ECLE with FGF2-SAP injected into the capsular bag (300 μl of a 1000 nM solution), the iris is homogeneously depigmented. (B) Three weeks after ECLE with rFGF2-SAP injected in the capsular bag (300 μl of a 1000 nM solution), the complete loss of iris pigments leads to a blue iris.

Explanted PMMA IOL
The IOLs were explanted and fixed in 4% paraformaldehyde, and the cells were stained with hemalum-eosin.

RESULTS
Slit Lamp Observations
Postoperative Inflammation. Nearly all the eyes—those injected with FGF2-saporin (either FGF2-SAP or rFGF2-SAP) and control eyes—used to evaluate
capsule opacification showed postsurgical inflammatory reactions. Those treated with Healonid were extremely inflamed and were not included in the evaluation protocol. For the other rabbits, there was moderate corneal edema, mostly in the superior part of the cornea, and fibrous deposits in the anterior chamber. The degree of corneal edema and fibrous exudation in rabbits given PMMA HSM IOL implants coated with FGF2-SAP or rFGF2-SAP and given control PMMA HSM IOL implants were the same. Thus, inflammation was not caused by the cytotoxin alone but was a result of surgery. Inflammation usually disappeared by 2 weeks after surgery, and the central area of posterior capsule was clear in most animals.

Rabbits injected intracameraly with only concentrated rFGF2-SAP (300 µl 1 µM solution) had severe stromal and subepithelial edema that led to neovascularization if it lasted more than 10 days (Fig. 1). The corneas of rabbits killed at this time were sectioned and fixed in paraffin for histologic examination to determine if endothelial cells were damaged.

Iris Changes. Rabbits given intracameral injections of FGF2-SAP showed loss of iris pigment and migration of the pigment to the posterior lens capsule 20 to 30 days after surgery. This phenomenon was much more marked in eyes injected with rFGF2-SAP; the iris color became entirely blue (Fig. 2). Pigmentation partly returned to irises 2 months after surgery.

Healonid. FGF2-SAP was mixed with Healonid (Pharmacia), and the mixture was injected into the eyes of six rabbits after ECLE of the lens. Two rabbits were administered 250 µl Healonid by injecting it into the capsular bag, two rabbits were administered 1.5 ng FGF2-SAP in 250 µl Healonid, and two rabbits were administered 14.4 ng FGF2-SAP in 250 µl Healonid. In vitro assays have shown that Healonid does not interfere with FGF2-SAP or with the cytotoxic effect of rFGF2-SAP on bovine epithelial lens cells. Inflammation was much more severe in this group of eyes than in the control eyes or in eyes injected with FGF2-SAP (in PBS), and they were therefore not included in the assessment. There was massive corneal edema the day after surgery, and peripheral revascularization developed rapidly because of the resultant elevated intraocular pressure. The same reaction occurred with Healonid alone and with FGF2-SAP in Healonid. Hence, the effect cannot be attributed to the drug alone.

Control Rabbits. Three rabbits had a ECLE with intracameral injections of FGF2 (300 µl of 10 nM solution). These rabbits showed few signs of inflammation, little fibrous exudate, and no corneal edema. FGF2 seemed to have an anti-inflammatory effect in the anterior segment. Neocrystallins had begun to form in the pupil area by 1 week after surgery. The rabbits given saporin injections into the capsular bag or implanted with saporin-treated IOLs had the same reactions after surgery that control animals had.

Macroscopic and Microscopic Appearance of the Capsule 24 Hours After Extracapsular Lens Extraction

Photographs were taken of the capsular bag with the IOL inside 1 day after ECLE to ensure that the protein seen during the following weeks did not result from reabsorption of cortical mass left in the capsular bag after extraction of the lens. Capsular bags and capsules were clear, and there was no macroscopic residual lens cortex. The histology of capsular bags from two rabbits 24 hours after they had undergone simple ECLE also showed no residual lens cortex and only a few residual lens fibers cells and equatorial potentially proliferative epithelial lens cells (results not shown).

Evaluation of Lens Regrowth

Extracapsular Lens Extraction With FGF2-SAP Injection. The posterior capsules could be seen in all rabbits once the fibrous membrane was reabsorbed (15 to 20 days after surgery). The eyes of seven control animals and eight rabbits treated by ECLE and intracapsular bag injection of FGF2-SAP were examined by a slit lamp (Table 2) and photographed 1 month (25 to 45 days) after surgery. There were de novo crystallins in five control rabbits, but there was slight clouding in some capsule fields in the remaining two. Four of the treated rabbits showed slight clouding in some fields, two rabbits had clear capsules, and two rabbits showed slight opacification (Fig. 3).

Part of the capsular opacification in the treated group was caused by pigment deposits on the anterior
FIGURE 4. Micrographs of the central area of the posterior lens capsule 2 months after ECLE. The capsule bags were excised, fixed, and stained with hemalumo-sine for light microscopy. (A) ECLE control. Multilayered cells and extracellular matrix. (B) ECLE with FGF2-SAP injected into the capsular bag; only few cells were alive in the center of the posterior lens capsule. (C) ECLE with HSM IOL, intracapsular bag implantation, multilayered cells, and extracellular matrix deposition. (D) ECLE with FGF2-SAP-coated HSM PMMA IOL implantation. The posterior capsule is clear with only some sparse cells.

side of the posterior capsule coming from iris pigments or pigmented anterior uvea cells. There was less capsule opacification in treated rabbits than in control rabbits (unpaired t-test, \( P < 0.01 \)). Capsular bags were fixed and stained (Fig. 4). Control capsules had multiple-layer deposits of cells with dense, translucent extracellular matrices. Cells in treated capsules usually appeared in a single layer, and there was a considerable amount of cell debris and pigment. In some areas, there were aggregates of pigmented cells.

**Extracapsular Lens Extraction With FGF2-SAP HSM PMMA IOL Implantation.** Capsule clouding was evaluated in nine control and nine treated rabbits 1 month after surgery (Fig. 5). Five of nine control animals had neocrystallins in the pupillary area, two had central, dense capsule opacification, and two capsules were slightly opaque. None of the treated rabbits had neocrystallins in the pupillary area, two had clear capsules, three had slight opacity in the periphery of the pupillary area, and four had central fibrosis. Capsular clouding also was evaluated 3 months after surgery. All seven control rabbits had dense capsule opacification, and five of seven had neocrystallins in the pupillary area. Four of six treated capsules were clear in the center, and the other two had fine fibrosis. There was significantly less lens regeneration in the treated rabbits than in the control rabbits 1 and 3 months after treatment (\( P = 0.0003 \) and \( P < 0.0001 \), respectively; unpaired t-test).

**Immunolocalization of FGF2-Saporin**

Sections of eyes 1 day after surgery (ECLE with FGF2-SAP injection) showed immunostaining for saporin on the anterior side of the posterior lens capsule, inside the epithelial lens cells, and in the intercellular space of the capsular bag. The toxin was still detected on the posterior lens capsule 5 days after surgery, but there was nonspecific immunostaining of inflammatory proteins (usually fibrin). Saporin was not detected 15 days after surgery (Fig. 6). There was also specific immunostaining in the ciliary body epithelium and in the iris epithelium and stroma 24 hours after surgery, but there was no signal on the corneal endothelium, the choroid, or the retina (Fig. 7). No specific immunostaining was found in either nonimmune serum control or in uninjected control eyes.

Sections of whole ocular globes that had undergone ECLE with FGF2-SAP-coated HSM IOL intracapsule bag implants were labeled specifically for saporin on the posterior capsule and inside the epithelial cells and in the iris stroma but not on the corneal endothelium, the choroid, or the retina. Eyes implanted with HSM IOLs coated with FGF2-SAP showed much less
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FIGURE 5. Clinical evaluation of lens regeneration in ECLE with FGF2-SAP-coated intracapsular bag implantation. (A) One month after surgery, capsule opacification was reduced significantly in the treated group compared to the control group (unpaired t-test, \( P = 0.0003 \)). Control rabbits underwent ECLE with ordinary HSM PMMA IOL implantation. (B) Three months after surgery, capsule opacification was reduced significantly in the treated group compared to the control group (unpaired t-test, \( P < 0.0001 \)). Control eyes underwent ECLE with ordinary HSM PMMA IOL implantation.

Histology of Tissue Samples

Tissue From Eyes That Had Undergone Extracapsular Lens Extraction Plus Injection of FGF2-SAP. The corneal endothelium showed no evidence of cell loss or lesion immediately after surgery or 1, 5, 15, or 30 days later. The corneal stroma was initially thickened because of edema caused by transitory endothelial cell dysfunction. There was no evidence of epithelial or stromal cell loss in the ciliary body. There was loss of iris epithelial and iris stromal pigment with a massive melanophagic reaction, but there was no evidence of mitosis or cell destruction in the iris epithelium. The histology of the retina and choroid plexus was normal.

Tissue From Eyes That Underwent Extracapsular Lens Extraction Plus Injection of rFGF2-Saporin. There was pronounced corneal edema 5 days after surgery and occasional loss of endothelial cells 2 months after surgery. The histologic findings were the same for the fusion protein and the disulfide-linked conjugate, but the reaction to the genetically engineered rFGF2-SAP was more pronounced, and there was severe loss of stromal pigment from the anterior stroma and the posterior pigmented epithelium. Large melanophagic cells full of pigment were present in the anterior part of the iris stroma. Iris structures were not inflamed. Ciliary body epithelium also seemed to be less pigmented than in the control rabbits. Pigmentation of the choroid was normal, and the structure of the retina and the morphology of its cells were unchanged.

Tissues From Eyes That Underwent Extracapsular Lens Extraction Plus FGF2-SAP and Hyaluronate Injection. Severe corneal edema and peripheral neovascularization developed. The ocular angle was stretched, indicating severe, persistent elevated intraocular pressure.

Tissues From Eyes That Underwent Extracapsular Lens Extraction With FGF2-SAP-Coated HSM IOL Implantation. Corneal edema developed 5 days after surgery, but there was no loss of endothelial cells from either treated or control implanted eyes. The corneal architecture was still normal 2 months after surgery. The structure of the iris was normal, but the anterior part

specific immunostaining on the iris than in eyes injected with FGF2-SAP (Fig. 8).

Histology of Tissue Samples

Tissue From Eyes That Had Undergone Extracapsular Lens Extraction Plus Injection of FGF2-SAP. The corneal endothelium showed no evidence of cell loss or lesion immediately after surgery or 1, 5, 15, or 30 days later. The corneal stroma was initially thickened because of edema caused by transitory endothelial cell dysfunction. There was no evidence of epithelial or stromal cell loss in the ciliary body. There was loss of iris epithelial and iris stromal pigment with a massive melanophagic reaction, but there was no evidence of mitosis or cell destruction in the iris epithelium. The histology of the retina and choroid plexus was normal.

Tissue From Eyes That Underwent Extracapsular Lens Extraction Plus Injection of rFGF2-Saporin. There was pronounced corneal edema 5 days after surgery and occasional loss of endothelial cells 2 months after surgery. The histologic findings were the same for the fusion protein and the disulfide-linked conjugate, but

FIGURE 6. Immunolocalization of FGF2-SAP on capsular bag sections 1, 5, and 15 days after ECLE and FGF2-SAP injection into the capsular bag. One day after surgery, a linear fixation of goat anti-saporin was observed on the anterior side of the posterior lens capsule. Five days after surgery, fixation still was observed on the capsule. Nonspecific fixation was caused by inflammatory proteins. Fifteen days after surgery, there was no fixation. FS 1000 = ECLE with FGF2-SAP (300 \( \mu l \) FGF2-SAP 1000 nM) injected into the capsular bag; C = ECLE with BSS injected into the capsular bag; D = day.
FIGURE 7. Immunolocalization of FGF2–SAP 1 day after ECLE with FGF2–saporin injected into the capsular bag. FGF2–saporin was detected on the capsule, the iris, and the ciliary body. No localization is observed on the corneal endothelium nor in the posterior segment of the eye. FS 1000 = ECLE with FGF2–SAP (300 μ FGF2–SAP 1000 nM) injected into the capsular bag; C = ECLE with balanced salt solution injected into the capsular bag; CE = corneal endothelium; cb = ciliary body; i = iris; c = capsule; r = retina.

of the stroma was depigmented; additionally, there were big, round cells full of pigment and dendritic melanocytes containing fine, pigmented granules (Fig. 9). The pigmented epithelium of the posterior side of the iris was normally pigmented (Fig. 9). The pigmented epithelium of the ciliary body had a normal structure and pigmentation. The choroid and retina had no lesions.

Tissues From Eyes That Underwent Extracapsular Lens Extraction With rFGF2–SAP-Coated HSM IOL Implantation. There was loss of corneal endothelial cells in some samples 2 months after surgery. There was a massive loss of pigment, but no loss of cells, from both the anterior stroma and the posterior epithelium of the iris. There appeared to be no lesions in the ciliary body, the retina, or the choroid.

Tissue Samples From Control Eyes That Underwent Extracapsular Lens Extraction With HSM IOL Implantation. Corneal edema developed in these eyes 5 days after surgery, but there was no cellular change or loss. The histology of the cornea was normal 2 months after surgery. The anterior part of the iris stroma lost pigment, but there were no changes in the other ocular tissues.

Macroscopic Appearance of the Capsular Bags 2 Months After Intraocular Lens Implantation
Retro-illuminated (back-lit) pictures taken in situ during excision and after excision showed a neo-lens surrounding the IOL in the controls and little protein synthesis in FGF2–SAP-coated IOL or rFGF2–SAP-coated IOL (Fig. 10).

Electrophoresis of the De Novo Soluble Proteins From the Capsular Bags
The normal lens was used as control for the synthesis of rabbit crystallins. Lanes for the treated and the control ECLE rabbits contained the same amounts of protein. All the samples had the same migration profile, indicating crystallins of molecular mass 20 to 40 kDa (results not shown). This confirmed that the newly synthesized proteins were mostly crystallins, as referred by Gwon et al.16 We, therefore, used the protein assay to measure lens regrowth.

Assay of Soluble Proteins From the Capsular Bag
Two months after surgery, the treated groups had approximately 8 times less soluble protein than the control group (P = 0.0024 for FGF2–SAP; P = 0.0147 for rFGF2–SAP; Mann–Whitney test). Three to 4 months after surgery, the amount of protein still was reduced significantly in the treated group compared to the control group (P < 0.001) (Fig. 11).

Photographs of Explanted Intraocular Lenses
The FGF2–saporin-coated (both FGF2–SAP and rFGF2–SAP) HSM PMMA IOL had many fewer cells and fewer protein deposits than the control HSM PMMA IOL (Fig. 12).

DISCUSSION
Rabbits frequently have been used for studies on lens regrowth. They are easy to care for and house, they have eyes that are not much smaller than human eyes, and the capsule clouds rapidly.17,18 Opacification of the human capsule is mostly caused by the migration of residual lens epithelial cells and their proliferation to produce fibroblastic metaplasia on the posterior lens capsule.19–21 The regeneration of the rabbit lens differs from human capsule opacification for two reasons. First, rabbit epithelial lens cells can synthesize
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FIGURE 8. Immunolocalizat-
Figure 8. Immunolocalization of FGF2-SAP 1 day after ECLE with FGF2-SAP coated HSM PMMA IOL in tracapsular bag implantation. A linear fixation was observed on the posterior lens capsule and on the posterior side of the iris adjacent to the IOL. FS 1000 ECLE with FGF2-SAP coated HSM PMMA IOL; C = control ECLE with simple HSM PMMA IOL; c = capsule; i = iris.

more neo-crystallins than humans21 (Fig. 13). Second, both epithelial lens cells and pigmented uveal cells proliferate in rabbits.22 Surgery also induces excessive fluid production in rabbits, with invasion of the anterior segment by protein and cells, which may contribute to capsule cell proliferation. The infusion of heparin during surgery can reduce this fibrous reaction, but heparin was not used because FGF2-saporin binds to it.23 These points should be taken into account when interpreting these results.

FGF2-SAP effectively inhibited lens regrowth when it was injected directly into the capsular bag (Figs. 3, 4). Four to 5 weeks after surgery, lens regrowth already began in control eyes. If there was direct injection of the cytotoxin into the capsular bag, no IOL was implanted and the anterior capsulotomy was performed by a linear incision; therefore, no anterior capsulectomy was realized. The same surgical procedure was performed in control and in treated eyes. FGF2-SAP injection in the capsular bag was efficient to prevent the lens regrowth. Immunolocalization demonstrated that FGF2-SAP injected into the anterior compartment binds to the lens capsule, inside the capsular bag, and remains there for at least 5 days. FGF2-SAP also is bound to epithelial lens cells, but it was never found in the posterior segment of the globe. There was no histologic evidence of loss of corneal endothelial cells, changes in the ciliary body alteration, or retinal toxicity. There were only changes in iris stromal and epithelial pigmentation without any loss or proliferation of iris epithelial cells. The fixation of FGF2-SAP on iris and ciliary body epithelium is not surprising because endogenous FGF2 has been localized in these tissues, suggesting that epithelial cells have high- and low-affinity FGF2-receptors.24 It is possible that the internalization of FGF2-saporin by the receptor pathway impaired the compartmentalization of the pigment organellosomes in the cells. The resultant shed pigment may be engulfed by neighboring melanophages or it may be released in the aqueous humor and deposited on the lens capsule or the IOL. Further experiments on the effect of FGF2-saporin on pigmented cells in vitro may help to solve these questions. The genetically engineered fusion protein caused much more iris reaction than the disulfide-linked one. Uneven pigmentation reappeared 2 months after treatment, indicating that the toxicity was transient. Pigmentation of the ciliary body epithelium also was altered, and pigment deposits on the posterior capsule contributed to a loss of capsule transparency in some rabbits.

There is no inherent reason why the two conjugates should have different toxicities. Rabbits are known to be extremely sensitive to endotoxin, and the proteins used in these studies were not endotoxin free. Thus, endotoxin could have caused inflammation, as did the surgical procedure.

FGF2-saporin-coated (either FGF2-SAP or rFGF2-SAP) HSM IOL implantation probably limited the binding of the cytotoxin to unwanted sites, including heparan sulfate, especially on the iris epithelium and stroma and on the ciliary body. Clinically, it did not modify postoperative inflammation. Intraocular lenses that were rinsed after incubation in FGF2-sa-
crystallins were seen in the pupillary areas of rabbit eyes implanted with coated IOLs, whereas there was an immediate and rapid production of lens crystallins in eyes implanted with uncoated IOLs. Photographs are not always as representative as slit lamp examination because the newly synthesized protein is transparent (Figs 13, 14). The amount of soluble protein (mostly crystallins) in eyes implanted with treated IOLs was highly reduced compared to the control eyes 6 weeks after surgery. The de novo synthesized proteins were rapidly observed in the pupil, and lens regrowth was usually not limited by synechiae between the anterior capsule and the IOL or by posterior synechiae. HSM PMMA IOLs have, in fact,

FIGURE 9. Iris histologic change 3 weeks after ECLE with rFGF2-SAP injected into the capsule bag (300 μl 1000 nM rFGF2-saporin). There seemed to be a little degree of artefactual shrinkage of the posterior layer of the iris in the top figure. (A) Control iris, original magnification ×100. (B) Iris loss of pigment in the epithelium and in the anterior stroma associated with a melanophagic reaction in the treated eye. Original magnification, ×200.

porin caused no loss of iris pigment, but IOL that were not rinsed caused loss of pigment at the iris rim. A zone of iris depigmentation was seen adjacent the capsular bag during dissection of the ocular globe. Immunohistochemistry showed FGF2-SAP bound to the posterior iris epithelium. Explanted FGF2-saporin-coated IOL removed 2 months after surgery had fewer cell and protein deposits than did uncoated IOL. Thus, heparin surface-modified IOL can be used to deliver FGF2-saporin to the posterior capsule and the epithelial lens cells after extracapsular extraction of the lens.

The clinical evaluation of lens regeneration is highly significant because no de novo synthesized

FIGURE 10. Pictures of capsular bags 3 months after ECLE with IOL implantation. (A) ECLE with FGF2-SAP-coated HSM PMMA IOL intracapsular bag implantation. The capsule was clear, and there were no crystallin proteins. (B) ECLE with ordinary HSM PMMA IOL intracapsular bag implantation. The capsular bag was full of lens material representing neo-synthesized proteins surrounding the IOL.
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Results for control eyes treated by saporin injection alone, or operated on and treated with saporin or FGF2, confirm that saporin is effective only when bound to FGF2. The fact that the heated protein has no effect suggests that the saporin acts only once it is bound to FGF2 receptors. Simulation of protein synthesis by FGF2 in rabbits confirms that fibroblast growth factors have a great influence on lens cell proliferation and differentiation.

Because mechanical cleaning of the capsular bag never is effective enough to eliminate all potentially proliferating epithelial lens cells, many pharmacologic agents have been used to inhibit lens cell proliferation. Most of the antimitotic drugs inhibit the proliferation and migration of lens epithelial cells in vitro. Daunomycin is the most promising, because it has no detectable toxic effects, and it prevents vitreoretinal proliferation in humans. In the first human clinical assays of the prevention of capsule clouding, daunomycin seemed to reduce lens cell proliferation and the formation of Elshnig's pearls, but did not prevent capsule fibrosis or capsule wrinkling. The ability of FGF2-saporin to prevent capsule clouding seems to be similar to that of daunomycin, but FGF-saporin has the advantage of being more specific for its target cells. Moreover, it can be delivered by heparin surface-modified IOL.

rFGF2-SAP may prevent capsule opacification in humans. Because it is specific for cells bearing the FGF2 receptor and it efficiently blocks protein synthesis, it is a good candidate for inhibiting the prolifera-
FIGURE 13. Photographic chart of the posterior capsule opacification graduation.

FIGURE 14. Slit lamp photographs. (A) Extracapsular lens extraction (ECLE) control 2 months after surgery. (B) ECLE with FGF₂-SAP (300 ml FGF₂-SAP 1000 nM) injected into the capsule bag 2 months after surgery. (C) ECLE with HSM PMMA IOL 2 months after surgery. (D) ECLE with FGF₂-SAP coated HSM PMMA IOL 4 months after surgery. (E) ECLE with HSM PMMA IOL 4 months after surgery. (F) ECLE with FGF₂-SAP-coated HSM PMMA IOL 4 months after surgery.
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Inhibition of epithelial lens cell proliferation and the synthesis of extracellular matrix on the posterior capsule. These experiments on rabbits show that coating HSM IOLs with FGF2–saporin (either FGF2–SAP or rFGF2–SAP) may be a good way of delivering the lowest efficient amount of toxin and reducing the risk of toxicity. Further experiments, including ultrastructural analysis of endothelial cells and electrophysiologic studies on the retina, are needed to ensure that FGF–saporin is not toxic for other ocular cell functions.

Key Words
fibroblast growth factor 2–saporin, secondary cataract inhibition

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