Iris Pigment Epithelial Translocation in the Treatment of Exudative Macular Degeneration

A 3-Year Follow-up

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Objective: To report the functional and anatomical outcome of 20 patients who underwent surgical removal of choroidal neovascularization combined with transplantation of autologous iris pigment epithelial cells to the subretinal space 3 years after treatment.

Methods: Freshly isolated autologous iris pigment epithelial cells were translocated to the subretinal space in 20 patients after membrane extraction. Patients were followed up by funduscopy, angiography, microperimetry, and visual acuity testing.

Results: After a follow-up of 3 years, 1 patient showed improved visual acuity, 13 patients retained stable visual acuity, and 3 patients had reduced visual acuity. No macular edema or recurrent choroidal neovascularization was apparent at any time during the follow-up.

Conclusions: Transplanted autologous iris pigment epithelial cells were well tolerated for 3 years and stabilization of visual acuity was achieved in most patients. These results suggest that iris pigment epithelial cells may serve as a substitute for retinal pigment epithelial cells after choroidal neovascularization removal in patients with exudative macular degeneration; however, whether these cells will be of any value for the restoration of vision and possible protection against choroidal neovascularization recurrence awaits further clinical observation and additional research.
harvest and because autologous iris pigment epithelial (IPE) cells can be easily obtained, it has been suggested that IPE cells may substitute for RPE cells for transplantation subretinally to replace missing or damaged RPE cells. During the last few years, several investigators have demonstrated that RPE and IPE cells have many important properties in common, such as pigmentation, cellular morphologic features, and formation of tight junctions. It has been shown that human, porcine, and rat IPE cells grown in vitro acquire the ability to phagocytose photoreceptor outer segments, which is a specific property of RPE cells in situ. In rabbits autologous IPE cells can be transplanted to the subretinal space, where they form a monolayer on top of the original RPE, phagocytose photoreceptor outer segments, develop microvilli, establish contact with the photoreceptor outer segments, and show no evidence of rejection during a 20-week follow-up. Recent studies have demonstrated that IPE cells may substitute for RPE cells for transplantation subretinally to replace missing or damaged RPE cells. During the last few years, several investigators have demonstrated that RPE and IPE cells have many important properties in common, such as pigmentation, cellular morphologic features, and formation of tight junctions.

**METHODS**

**PATIENTS**


**MEDICAL AND OPHTHALMIC EXAMINATION**

To establish a baseline, all patients underwent a comprehensive ophthalmologic examination preoperatively, including anterior segment biomicroscopy, intraocular pressure measurement, and fundus examination with slitlamp biomicroscopy. Best-corrected distance VA was measured by a certified visual examiner (S.A.) with the use of the Early Treatment Diabetic Retinopathy Study (ETDRS) chart according to a fixed protocol. For each vision test, both eyes of the patient were tested with different charts. Color photographs of the macula and the optic disc of each eye were taken; fluorescein and indocyanine green angiograms were performed by a certified photographer. The size of CNV was measured in late-phase fluorescein angiograms; measurements were taken in triplicate and the CNV size is given as disc area in accord with the Macular Photocoagulation Study Group. The size of CNV was measured in 10 of 20 patients; for the remaining 10 patients either the angiograms were unavailable or the angiograms did not allow for accurate measurement because of cloudy media or quality of the angiogram. Microperimetry of the study eye was performed using scanning laser ophthalmoscopy (SLO II; Rodenstock, Munich, Germany). Patients had a complete medical history, physical examination, electrocardiogram, chest radiogram, and blood test to screen for major organ pathologic conditions.

**SURGICAL TECHNIQUE**

**Cell Isolation**

A large iridectomy was performed on the eye undergoing vitrectomy through a peripheral corneal incision. The tissue was placed in a glass well with a drop of balanced salt solution (BSS; Alcon Pharma GmbH, Freiburg, Germany) and the IPE cells were mechanically isolated from the underlying stroma without the use of enzymes to avoid possible damage and alteration of the cells. For injection the cells were suspended in 20 µL of balanced salt solution.

**Neovascular Membrane Removal and Cell Transplantation**

In all patients a 3-port pars plana vitrectomy was performed. A small retinotomy was made superior or temporal to the fovea; the fibrovascular membrane was seized with forceps and extracted slowly through the retinotomy. If necessary, the bleb retinal detachment was enlarged by injecting a stream of balanced salt solution. The IPE cells (approximately 12,000 cells per eye) were injected into the subretinal space using a Hamilton syringe fitted with a glass pipette tip. The syringe, which had been coated with autologous serum prior to filling it with IPE cells, was introduced and positioned just over the retinotomy. After the cells were injected, a fluid-air or a fluid-gas exchange was performed. In one case cataract surgery prior to iridectomy and vitrectomy was performed. Choroidal neovascular extraction and IPE transplantation was performed by 3 different surgeons (P.W., P.E., and K.U.B.-S.) following identical surgical protocol and technique.

**PATIENT FOLLOW-UP**

All patients were scheduled for examination at 3 weeks and at 3, 6, 12, and 36 months. The 36-month examination took place between 36 and 48 months. Postoperative ophthalmic examinations included all the parameters of the preoperative examination. Three-year follow-up was completed in 17 patients; 3 patients were unavailable for follow-up at 3 years—2 patients died of diseases unrelated to the treatment and 1 patient was unable to attend the scheduled visit because of severe heart disease. All 3 patients lost to follow-up belonged to the group with classic CNV secondary to AMD.

**STATISTICAL METHODS**

The primary efficacy outcome was change in VA after 3 years (logarithm of the minimum angle of resolution [logMAR] and
Snellen equivalent) of the operated-on eye compared with baseline VA. Data were described by mean (SD) and frequencies, as well as corresponding 95% confidence intervals. Detailed comparisons were performed using 95% confidence intervals corresponding to the least significant difference t test. Effects are considered statistically significant if the P value of the corresponding test drops below .05. To study the possible bias resulting from the missing observations at the 3-year follow-up visit, 2 populations were investigated. First, the data from the full analysis set with complete observations (n=17) were evaluated. Next, data from the full analysis set using missing observation substitution (last observation carried forward) (n=20) were evaluated. Computations were performed using SAS software (SAS Institute Inc, Cary, NC) under MacOS (Apple Computers, Inc, Cupertino, Calif).

### RESULTS

#### BASELINE CHARACTERISTICS AND FOLLOW-UP

Submacular surgery with CNV extraction was performed in 20 eyes of 20 patients; autologous IPE cells were transplanted in 19 of 20 patients. In 1 patient the retinal bleb could not be enlarged sufficiently to inject the cell suspension. The series consisted of 17 female and 3 male patients who had a median age of 67.9 years (age range, 33-85 years). Follow-up ranged from 36 to 48 months (mean, 40.2 months). Baseline characteristics and VA data of these patients are summarized in the Table. Preoperative best-corrected VA ranged from 20/1000 (logMAR, 1.7) to 20/125 (logMAR, 0.8), with a mean of 20/320 (logMAR, 1.2). Preoperative fluorescein angiography showed classic subfoveal CNV due to AMD in 9 eyes and occult subfoveal CNV in 7 eyes. The eyes of 4 younger patients showed either idiopathic CNV or CNV associated with dominant drusen or pathologic myopia. Choroidal neovascularization size ranged from 0.8 to 10.7 disc diameters, with a median of 4.1 disc diameters (Table). Functional outcome did not correlate with lesion size in these patients.

#### VISUAL OUTCOMES

Three years after IPE transplantation, best-corrected VA ranged from hand movements (logMAR, 0.7) to 20/100 (logMAR, 0.7), with a mean of 20/320 (logMAR, 1.2). Compared with preoperative VA, 1 patient showed improvement of 3 lines or more on the ETDRS chart, 13 patients retained stable VA with a change of less than 3 lines, and 3 patients showed decreased VA of 3 lines or more (Table). Of the 3 patients who showed decreased VA, 1 patient had proliferative vitreoretinopathy necessitating 2 revisional vitrectomies.

#### ANGIOGRAPHIC OUTCOMES

In fundus photographs transplanted cells appeared as dark spots in the area of the transplantation that appeared to remain stable in size and shape throughout the observation period (Figure). Throughout this 36 to 48 months’ follow-up period, the adjacent retina did not show evidence of immunologic rejection, such as inflammatory reactions with destruction of the transplanted cells or macular edema. Postoperative angiograms did not show signs of recurrence of the CNV at any time in any of the patients (Figure). None of the patients showed central fixation or responses over the transplanted areas using macular perimetry 3 years after submacular surgery.

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<table>
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<tr>
<th>Patient No./Sex/Age, y</th>
<th>CNV</th>
<th>Visual Acuity</th>
<th>Follow-up, mo</th>
<th>Difference in Visual Acuity, No. of Lines</th>
<th>CNV Size, Disc Diameter*</th>
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<tr>
<td></td>
<td></td>
<td>Baseline</td>
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<td>At 3 mo</td>
<td>At 6 mo</td>
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Abbreviations: AMD, age-related macular degeneration; CNV, choroidal neovascularization; HM, hand movements; NA, not applicable.

*Choroidal neovascularization size given as standard disc areas as published by the Macular Photocoagulation Study Group.*
COMPLICATIONS

All 19 phakic patients developed cataract on the operated-on eye and underwent cataract surgery; in 1 case phacoemulsification was combined with the initial vitrectomy and IPE translocation. Two patients experienced significant complications, namely, retinal detachment and proliferative vitreoretinopathy, during the first 2 months postoperatively and underwent revision vitrectomy with silicone oil tamponade at 3 months. The eye that could not be successfully injected with IPE cells developed a macular pucker, and that patient underwent revision vitrectomy with epiretinal membrane peeling 5 months after the initial surgery.

STATISTICAL ANALYSIS

The main criterion of analysis was comparison of change in VA (logMAR) after 3 years vs baseline. Mean change in VA (logMAR) considering the 17 patients who completed the 36 months’ follow-up was not significant; mean change in all 20 patients substituting missing values by last observation carried forward showed the same tendency. In a 3-factor repeated-measures analysis of variance model with all factors treated as continuous, VA showed no dependency from age. However, logMAR values increased linearly, depending on baseline values \((P<.001)\), and slightly over time.

COMMENT

The development of neovascularization may lead to irreversible damage to the RPE and photoreceptors, and removal of the membranes is associated with the traumatic loss of the RPE cell layer in AMD. Photodynamic therapy as the current standard in the treatment of at least predominantly classic subfoveal CNV in AMD can stabilize VA in most patients without the risk of surgery associated complications and adverse effects. However, only a few patients with exudative maculopathy are eligible for the treatment with photodynamic therapy.\(^6\,44,45\) Furthermore, a considerable number of re-treatments are necessary because of recanalization or recurrence of the CNV. Surgical removal of the neovascular complex is technically feasible in almost all patients with exudative AMD; but the procedure is usually associated with traumatic loss of the RPE cell layer in AMD.\(^46,47\) Most studies that investigated the effect of submacular surgery in exu-
dative AMD have concluded that VA in the best case can be stabilized or slightly improved after membrane extraction. To improve postoperative VA, membrane extraction should be accompanied by restoration of the diseased and surgically damaged Bruch membrane–RPE complex.\textsuperscript{14,15}

Gouras et al\textsuperscript{48} postulated that RPE cell translocation to the area of RPE loss would reestablish functional and morphologic integrity of the retina-photorceptor complex thus improving or restoring vision. Algvere et al\textsuperscript{19,20} transplanted human fetal RPE cells in patients with non-exudative and exudative AMD; however, the transplanted cells did not survive and proliferate, due to rejection of the allograft, insufficient attachment to the Bruch membrane, or other unknown causes. Algvere et al also demonstrated that transplantation of homologous adult human RPE cells to the subretinal space is feasible and that in patients with an intact blood-retinal barrier, namely, patients with dry AMD, the transplanted RPE cells survive for up to 12 months without adversely affecting the photoreceptors. To prevent rejection it would be necessary to transplant autologous RPE cells or to immune suppress the host after transplantation of allogeneic RPE cells. The feasibility of isolating and transplanting autologous RPE cells at the time of CNV excision has been demonstrated by Binder et al\textsuperscript{24} in a trial with 20 patients, who have been followed up for 12 months. Recently van Meurs et al\textsuperscript{49} published the results of a series of 8 patients treated with a modified technique of autologous RPE cell transplantation. However, the routine isolation and transplantation of viable autologous RPE cells at the time of CNV excision is challenging. Full-thickness transplants that were taken from areas adjacent to the CNV showed sequestration after a longer observation period.\textsuperscript{22,25} Furthermore, proliferative vitreoretinopathy rates, as one of the main surgery-associated complications, have been relatively high in these first case series.\textsuperscript{24,49} Because autologous RPE cells are difficult to obtain in a sufficient number of viable cells and possibly carry the same defect as the subfoveal diseased RPE, it may be preferable to transplant autologous IPE cells that hypothetically could acquire RPE functions in the subretinal space. The hypothesis that IPE cells may acquire RPE functions in the subretinal space is supported by experiments in which IPE cells were transplanted as a suspension to the subretinal space of rabbits and of Royal College of Surgeons (RCS) rats. In a 20-week study, IPE cells transplanted to the subretinal space of rabbits survived without aid of immunosuppressive agents, formed a single layer of cells between the RPE and the photoreceptor cells layer, developed microvilli, attached to the retina and to the RPE layer, and demonstrated phagocytosis of photoreceptor outer segments without disturbing the morphology of the photoreceptor layer.\textsuperscript{34} In the RCS rat, which is a model of AMD, transplanted IPE cells demonstrated the same characteristics and in addition prevented photoreceptor degeneration.\textsuperscript{33,34,37,38} Several studies examined the feasibility of transplanting autologous IPE cells to the subretinal space.\textsuperscript{34,35,37}

In this study, 1 of 17 patients who were examined 3 years or longer after autologous IPE transplantation showed significant improvement, and 13 patients showed stabiliza-

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REFERENCES