Human Neural Retinal Transplantation

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PURPOSE. A pilot study of human neural retinal transplantation was undertaken to investigate three major issues: whether a safe surgical procedure could be devised for transplantation of neural retinal tissue into the subretinal space, whether the transplant would be accepted in the subretinal space, and whether an improvement in vision could be achieved.

METHODS. Eight patients with bare light perception (LP) vision due to retinitis pigmentosa (RP) and one patient with bare LP vision due to advanced neovascular age-related macular degeneration (AMD) received subretinal transplants of human fetal retinal microaggregate suspensions without postoperative systemic immunosuppression. The patient with AMD also received a fetal retinal sheet transplant. The ages of the patients ranged from 31 to 94 years (median, 55 years). The preand postoperative evaluations included visual function testing, detailed fundus examinations, fundus photography, fluorescein angiography, macular perimetry using a scanning laser ophthalmoscope (SLO), and full field and focal electroretinograms (ERGs).

RESULTS. Three of the eight RP patients demonstrated possible improved light sensitivity during the initial months of follow-up. However, visual improvement disappeared between 3 and 13 months of follow-up. After transplantation, no subject showed any changes in the ERG recordings or SLO macular perimetry relative to their preoperative baseline. No patient experienced a retinal detachment, infection, or extensive bleeding. None of the patients developed retinal vasculitis or intraocular inflammation. In one RP patient, fluorescein angiography and fundus photography documented the formation and maturation of new host retinal vessels in the area of the transplant.

CONCLUSIONS. Transplantation of fetal retinal photoreceptor suspensions into the subretinal space was achieved safely in nine subjects. Although a definite positive effect on visual function could not be demonstrated, the apparent high tolerance for graft tissue is promising for future efforts in the field of neural retinal transplantation. (Invest Ophthalmol Vis Sci. 2000;41:3100–3106)

Retinal transplantation was first performed in 1946 by Tansley,1 who demonstrated features of retinal differentiation in embryonic ocular tissue transplanted into the brains of young rats. In 1959, Royo and Quay2 reported the first intraocular retinal transplantation procedure and demonstrated that fetal rat retina could survive in the anterior chamber of the maternal parent. However, significant interest in retinal transplantation was not generated until the mid-1980s. del Cerro et al.3 transplanted full-thickness strips of retina into the anterior chamber of a mouse and demonstrated survival of both allografts and xenografts. Turner and Blair4 transplanted neonatal rat retina into the subretinal space via a transscleral approach and demonstrated survival and differentiation of the graft into retinal layers. It was found, however, that survival of grafted tissue could be improved by using a younger donor.5

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we wanted to investigate whether a safe surgical procedure could be devised for transplantation of the neural retina into the subretinal space, whether the transplant would be accepted in the subretinal space, and whether there was any improvement in vision after the transplantation.

**METHODS**

In an ideal experimental design, there is only a single variable. Choosing a subject population to study the feasibility of retinal transplantation similarly should have the fewest variables possible. At the same time, ethical considerations direct us to choose as subjects patients who have little risk of being adversely affected by the experimental procedure, and who are well-informed of the risks and benefits of human retinal transplantation in its early stage of investigation. With these considerations in mind, we used the selection criteria set forth in Table 1.

Eight patients with end-stage retinitis pigmentosa (RP) with bare light perception (LP) and one patient with LP in one eye and no light perception (NLP) in the other eye, due to a large subretinal hemorrhage secondary to neovascular age-related macular degeneration (AMD), participated in our study. All subjects received subretinal transplants of human fetal neural retinal microaggregate suspensions without postoperative systemic immunosuppression (Table 2). The AMD subject also received a full-thickness sheet of retinal tissue. In all patients, the fellow eye was left untreated as a control.

All subjects volunteered to undergo the experimental procedure. Extensive discussion with the patients and their relatives took place before enrollment, emphasizing that in all likelihood the subject would not experience an improvement in their vision and that this was the first phase of an experimental protocol to determine safety primarily and efficacy only secondarily. The protocol was approved by the Johns Hopkins University School of Medicine Institutional Review Board, and a written and a videotaped informed consent were obtained for every subject. This study also adhered to the Recommendations Guiding Medical Doctors in Biomedical Research Involving Human Subjects provided by the Declaration of Helsinki.

***Table 2. JHU Neural Retinal Transplant Subjects: Results as of April 1, 1998***

<table>
<thead>
<tr>
<th>Pt</th>
<th>Age, y</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Eye</th>
<th>Preop Vision</th>
<th>Follow-Up Time, mo</th>
<th>Current Vision</th>
<th>Changes</th>
</tr>
</thead>
<tbody>
<tr>
<td>BC</td>
<td>33</td>
<td>M</td>
<td>Late-stage RP</td>
<td>OD</td>
<td>Medium light perc.,</td>
<td>14</td>
<td>Dim/med. LP, inf./side proj.</td>
<td>Slightly better (both eyes) None, then slightly worse</td>
</tr>
<tr>
<td>PG</td>
<td>55</td>
<td>M</td>
<td>Late-stage RP</td>
<td>OS</td>
<td>Medium LP, centr./</td>
<td>13</td>
<td>Medium LP, centr./far temp. proj.</td>
<td>None</td>
</tr>
<tr>
<td>RG</td>
<td>94</td>
<td>M</td>
<td>AMD, subret.</td>
<td>OS</td>
<td>Bright flash only, far</td>
<td>10 (deceased)</td>
<td>Bright flash only, far temp. proj.</td>
<td>None</td>
</tr>
<tr>
<td>BH</td>
<td>69</td>
<td>F</td>
<td>Late-stage RP</td>
<td>OS</td>
<td>Bright flash only</td>
<td>12</td>
<td>Bright flash only</td>
<td>None</td>
</tr>
<tr>
<td>LJ</td>
<td>31</td>
<td>F</td>
<td>Late-stage RP</td>
<td>OD</td>
<td>Bright flash only</td>
<td>5½</td>
<td>Medium LP, some inf./side proj.</td>
<td>Slightly better, but gain then lost None</td>
</tr>
<tr>
<td>RJ</td>
<td>62</td>
<td>F</td>
<td>Late-stage RP</td>
<td>OD</td>
<td>Medium LP, little or</td>
<td>10</td>
<td>Medium LP, some inf./side proj.</td>
<td>None</td>
</tr>
<tr>
<td>JL</td>
<td>52</td>
<td>F</td>
<td>Late-stage RP</td>
<td>OD</td>
<td>Bright flash only</td>
<td>5½</td>
<td>Bright flash only</td>
<td>None</td>
</tr>
<tr>
<td>VO</td>
<td>58</td>
<td>M</td>
<td>Late-stage RP</td>
<td>OS</td>
<td>Dim/med. LP, some sup./side proj.</td>
<td>11</td>
<td>Dim light perc., some sup./side proj.</td>
<td>Better, then lost (ctrl eye normal) None</td>
</tr>
<tr>
<td>CS</td>
<td>64</td>
<td>M</td>
<td>Late-stage RP</td>
<td>OD</td>
<td>Dim/med. LP, some inf./side proj.</td>
<td>13</td>
<td>Dim/med. light perc., some inf./side proj.</td>
<td>Ctrl not Rx eye, slightly worse</td>
</tr>
</tbody>
</table>

Note: dim LP is more sensitive than medium LP; subjects with better initial vision appear to fare best. ctrl, control; perc., perception; proj., projection; centr., central; inf., inferior; sup., superior; tmp., temporal.
Donor Tissue Isolation

All fetal tissue was obtained and used according to federal government guidelines for fetal tissue use and in accordance with the University of Rochester and the Johns Hopkins University Medical School Institutional Review Board guidelines. In particular, the possibility of donating tissue for transplantation was discussed with the donor only after the decision to terminate the pregnancy was made. Screening tests for HIV, human T-cell lymphotropic virus (HTLV-I/II), and hepatitis B/C pathogens were performed on the donor’s blood.

Fetal suspensions of neural retinas were obtained from the eye vesicles of 14- to 16-week-old fetuses after scheduled pregnancy termination. Tissue was obtained within 1 hour after surgery. The eyes were collected in Optisol-GS (Chiron Vision, Irvine, CA) at 4°C. The globes were hemisected and retinas were dissected free of the retinal pigment epithelium (RPE) in Optisol. The retinas as a whole were stored for 48 to 72 hours in Optisol at 4°C until transplantation. Viability was tested using the trypan blue (0.4%; Gibco, Grand Island, NY) exclusion method on a small tissue sample before transplantation. All transplants showed greater than 90% viability by this test.

Transplantation Procedure

Transplant procedures were the same for all subjects. Local retrobulbar anesthesia was obtained by injecting 5 ml of a mixture of 4% lidocaine and 0.75% marcaine into the retrobulbar space. A standard three-port pars plana vitrectomy was then performed. The fetal tissue was transferred from Optisol to Dulbecco’s modified Eagle’s medium (DMEM; Gibco) with 4.5 g/l of glucose. The microaggregate suspension was created by cutting the tissue into fragments smaller than 1 mm² in size using microscissors, followed by gentle aspiration and ejection of the fragments multiple times using a soft-tip cannula. The microaggregates were introduced into the subretinal space by piercing the retina with the soft-tip cannula, followed by injection of 0.1 to 0.2 ml of the suspension into the subretinal space. For the fetal sheet transplant placed in the eye of the patient with AMD, a piece of the 2 × 2 mm retina was cut with microscissors and then grasped with a smooth-tip custom-built microforceps. The tip of the forceps was used to pierce the retina and, after entering the subretinal space, the tissue was released such that the outer retinal layer was facing the host RPE.

For the eight RP patients, the microaggregate suspension was placed in the macular region. Because there was an extensive disciform scar in the macula of the AMD patient, both the microaggregate suspension and the retinal sheet were transplanted in an extramacular location superior to the optic nerve head.

Visual Function Testing and Clinical Examinations

To measure and monitor the severely reduced vision of the patients, four monocular assessment methods were used (Table 3).

Dark-Adapted Thresholds. After pupil dilation and 45 minutes of dark-adaptation, absolute flash thresholds were determined in the computer-controlled Ganzfeld bowl of a clinical ERG system (model UTAS 2000; LKC, Gaithersburg MD), using a two-alternative forced-choice procedure. The light intensity could be reduced in 2-dB steps from the 0 dB maximum of 2.6 candela (cd)/m², to the system’s −48 dB minimum of 42 μcd/s/m²; if the patient’s limited sensitivity did not allow perception of the brightest flashes, the photographic flash (Vivitar 283, Vivitan, Newbury Park, CA) in the Ganzfeld was used, allowing relative intensities up to +34 dB to be presented.

Light Projection. Using a penlight with reduced intensity, a crude dark-adapted confrontation field test was performed at 5 eccentricities and 12 clock hours.

Electroretinogram. Dark-adapted full field flash and flicker ERGs were recorded using the UTAS 2000 system and Burian–Allen electrodes (Hansen Labs, Iowa City, IA). Responses to flicker frequencies of 20, 25, 31, 35, and 40 Hz, recorded with 30-Hz high- and low-pass filtering, were Fourier-analyzed to allow response detection down to the noise level of 50 to 100 nV. After light-adaptation, a focal ERG was also performed, using LKC’s handheld ophthalmoscope stimulator.

Scanning Laser Ophthalmoscope (SLO). Projection and, if possible, crude form recognition were explored in the Rodenstock SLO (Canon USA, Lake Success, NY), at retinal illuminance levels up to 70,000 td.

In addition to visual function testing, the patients’ eyes were followed by serial ophthalmoscopic examinations, fundus photography, and fluorescein angiography. Patients were evaluated twice before surgery and then at 1 day, 1 week, and approximately 1, 2, 3, 6, 9, and 12 months after transplantation.

RESULTS

The age, sex, preoperative and follow-up vision, follow-up time, and changes of the 8 RP and 1 AMD subjects are summarized in Table 2. In the following sections, the clinical findings, psychophysical data, and electrophysiological test results are presented.

Clinical Appearance

Postoperatively, no subject showed any evidence of infection, retinal detachment, or rejection. There was no hypopyon,
increased vitreous inflammation, retinal vasculitis, or cystoid macular edema and neither were Dalen-Fuchs nodules present. Immediately after surgery, transplanted tissue was clearly visible as a whitish subretinal deposit (Fig. 1A). Fluorescein angiography showed retinal microvascular dilatations over the transplanted tissue (Fig. 1B). Late frames of the angiogram showed leakage primarily at the outer retinal level (Fig. 1C). By 3 months after surgery, the transplanted tissue became more transparent but continued to remain visible. In one of the subjects (VO), starting on postoperative day 3, new blood vessels began to develop from existing host retinal vessels. By

**FIGURE 1.** (A) Patient VO: Postoperative fundus photograph 3 days after transplantation. A retinal microaggregate suspension (0.2 ml) was placed in the subretinal space along the inferotemporal arcade. The graft at the inferotemporal arcade is marked with arrows. An air bubble meniscus is seen along the superotemporal arcade. (B) Fluorescein angiogram on day 3 after retinal transplantation. Thirty-three seconds after fluorescein injection, there is apparent vascular dilatation over the area of the transplant (arrow) with early leakage from these vessels. (C) The same angiogram 348 seconds after fluorescein injection shows deep retinal leakage in the area of the transplant.

**FIGURE 2.** (A) Fluorescein angiogram 3 months after transplantation in the eye of patient VO. Fifty seconds after fluorescein injection, there is evidence of new retinal vessels (arrows) in the area of the transplant at the level of the inner retina. The graft is less visible and now minimally blurs the details of the underlying choroidal vasculature. (B) The same angiogram 353 seconds after fluorescein injection shows superficial leakage over the area of new vessel growth, as well as leakage at the level of the RPE. This leakage is slightly greater than that seen on day 3 after transplantation.
the third postoperative month (Fig. 2A), these blood vessels developed into multiple neovascular fronds overlying the fetal microaggregate transplant. Some microscopic intraretinal hemorrhage was also present. Fluorescein angiography from this visit demonstrated leakage of dye, indicative of an immature blood–retina barrier (Fig. 2). There was, however, no staining of the vessel wall (to suggest vasculitis) or cystoid macular edema. Over the next 6 months, the new vessels lost their frondlike appearance and assumed a more “mature” configuration. By postoperative month 11 (Fig. 3), there was no evidence of retinal hemorrhage, and fluorescein angiography (Fig. 4) revealed no leakage from the vessels, suggesting the presence of an intact inner blood–retina barrier.

**Visual Function Tests**

**Dark-Adapted Thresholds.** Figure 5 shows flash detection thresholds as a function of time after surgery in each subject’s treated eye, relative to the thresholds in the untreated eye. Data are not shown for the AMD subject whose vision was bare LP vision only in far temporal projection (both before and after surgery) in the treated eye and NLP in the fellow eye. As a result, meaningful relative changes in threshold sensitivity cannot be presented for this subject. Two patterns can be seen. Short-term changes within 1 month after surgery characterized by a decrease in sensitivity (i.e., an elevation of the dark-adapted threshold) were noted in subjects PG, BH, JL, and CS. This decreased sensitivity remained stable in two patients (PG, JL), returned to the preoperative baseline in one (BH), and improved beyond the preoperative level in one (CS, see below). Long-term (1–3 months after surgery) changes characterized by an increase in sensitivity were evident in subjects RJ, VO, and CS. However, this positive effect gradually disappeared after 3 to 13 months in all subjects.

**Light Projection.** Due to subjects’ limited ability to maintain fixation, this test proved less reliable than the visual fields determined in the SLO.

**Electroretinography.** Reliable ERG recordings could be performed in six subjects; severe nystagmus in the remaining two preempted ERG signals of acceptable quality. In three subjects, no ERG responses were detected at any time. Of the remaining three, LJ and JL had recordable (100–200 nV; i.e., 0.1–0.2 μV) flicker ERGs in both eyes preoperatively, but lost the responses in the operated eye after transplantation; only BC retained a recordable (50–150 nV) flicker ERG in the operated eye. No recordable focal ERG responses were obtained.

**Scanning Laser Ophthalmoscope.** Three subjects were able to see bright fixation targets in the SLO, and the develop-
iodin has also been reported.23,24 Using techniques similar to and AMD in the Low Vision Service at our institute.29,30

logical tests to better define the visual status of the patients because RP subjects are known to have fluctuations in the authors themselves urge caution in interpreting these results performed over the area of the graft in these subjects, and the visual field on static perimetry. SLO macular perimetry was not observed transient improvement in dark-adapted sensitivity in two. This finding suggests that the transplant procedure itself can transiently disrupt the local visual function and electrical properties of the host retina. However, we also observed transient improvement in dark-adapted sensitivity in three subjects during the initial months of follow-up. This improvement disappeared by 3 to 13 months’ follow-up in all three subjects. Another five subjects (including the AMD subject) showed no improvement in their visual function. No improvements in SLO perimetry or the ERG recordings were found in any subject, including the three patients with improved light sensitivity. The failure to observe a change in retinal fixation patterns in the SLO perimetry in patients with increased light sensitivity suggests that the improvement was not due to the functional integration of the graft with the host. It is possible, however, that the graft exerted some humoral effect on surrounding areas of the host retina, thereby improving the light sensitivity.

Spontaneous fluctuation of vision in patients with advanced retinal diseases is quite common.30,31 As a result, transient changes in dark-adapted light sensitivity may not reflect an effect of the transplant itself. On the other hand, work in our department has demonstrated that in experienced subjects, this test can be highly reproducible.29 Consequently, changes of 10 dB or more (subjects VO, CS) in the dark-adapted light sensitivity data (presented as a relative change; i.e., treated eyes compared with nontransplanted fellow eyes, which served as controls) are almost certainly caused by real, albeit transient, changes. It should be noted that findings such as these only underscore the importance of further developing and validating precise and sensitive vision tests.

Although a persistent treatment benefit could not be established in this study, transplantation of fetal retinal tissue appears to be safe. No patient experienced a retinal detachment, infection, significant bleeding, or other complication after surgery. Furthermore, no patient demonstrated any clinical evidence of rejection; specifically, there was no evidence of intraocular inflammation, retinal vasculitis, or retinal edema. Subclinical “rejection,” or loss of graft tissue without a significant cell-mediated immune response, cannot be ruled out.

Our observations are similar to those of Kaplan and coworkers32 who transplanted vibratome-harvested adult photoreceptor cells into the subretinal space of two patients with advanced RP. Kaplan et al.32 reported no intraoperative or postoperative complications or clinical evidence of rejection but also observed no improvement in vision. However, visual acuity was only measured with an indirect ophthalmoscope; dark-adapted light sensitivity was not measured. Furthermore, adult tissue, rather than fetal retina, was the source of the donor tissue. A number of animal studies have shown that graft survival declines with increasing age of the donor.5

Das et al.53 also transplanted suspensions of fetal neural retinal microaggregates into the subretinal space of 14 RP subjects with bare LP in Hyderabad, India. The ERG remained unrecordable in all the subjects. Several reported subjective improvements in vision from LP to hand motions, and one subject reported regaining 20/200 Snellen acuity and a 3° visual field on static perimetry. SLO macular perimetry was not performed over the area of the graft in these subjects, and the authors themselves urge caution in interpreting these results because RP subjects are known to have fluctuations in the course of their illness.50,51

More recently, Radtke and coworkers34 transplanted intact sheets of fetal retina (15 and 17 weeks’ gestational age) into the subretinal space of two patients with autosomal recessive RP. Both patients reported a subjective improvement in vision after the transplant, and one patient showed a transient multifocal electroretinography (mERG) response in the trans-

FIGURE 5. Psychophysical flash threshold sensitivity data for the treated (transplanted) eyes of all subjects relative to the untreated fellow (control) eyes, before and after treatment. No values are shown for the single AMD subject, whose untreated fellow eye had NLP vision. Preoperative values are presented at abscissa value 0.

**DISCUSSION**

Animal experiments performed by multiple investigators over the last 15 years have demonstrated that neural retinal tissue can last and differentiate after transplantation to the subretinal space.3,6,12,14,16,27,28 Moreover, possible visual improvement as manifested by the modification of learned behavior has also been reported.23,24 Using techniques similar to those used in prior animal studies, we embarked on human retinal transplantation to address three questions: Is the surgical procedure safe? Is the transplant accepted in the subretinal space? Can improvement in vision be achieved and if so for what duration?

Although human retinal transplantation can answer these questions in the most direct fashion, ethical considerations of bringing no harm to a patient directed us to choose subjects who would have the least risk of being adversely affected by the transplant procedure (i.e., patients with bare LP or NLP vision). A potential disadvantage of choosing patients with severely reduced vision is that obtaining reproducible objective measurements of visual function can be difficult. For this reason, we used a series of psychophysical and electrophysiological tests to better define the visual status of the patients enrolled in this study. This battery of tests has been evaluated in a prospective fashion to demonstrate its reliability and reproducibility in patients with advanced vision loss due to RP and AMD in the Low Vision Service at our institute.29

After retinal transplantation, we observed a worsening of flash-threshold sensitivity in three patients and a loss of ERG responses in two. This finding suggests that the transplant procedure itself can transiently disrupt the local visual function and electrical properties of the host retina. However, we also observed transient improvement in dark-adapted sensitivity in three subjects during the initial months of follow-up. This
planted area at 4 months. However, the mfERG response could not be recorded at 6 and 9.5 months after transplantation. This transient response may be akin to the temporary improvement in light sensitivity observed in our study. In addition, the overall follow-up period for both patients was less than 14 months, and, thus, long-term effects of the transplant are unknown.

In summary, a persistent definite visual improvement was not observed in our study after transplantation of fetal neural retinal tissue into the subretinal space of eyes of patients with advanced RP or AMD. Although a small decrease in flash sensitivity was observed shortly after transplantation in a few patients, this decrease either improved or stabilized. However, the graft appeared to be well-tolerated, and, thus, this phase I trial does appear to establish the safety of fetal retinal transplantation. For transplantation to become a viable therapeutic option for patients with retinal degenerations, a number of obstacles will need to be overcome. New techniques will likely be required to allow larger quantities of tissue to be transplanted into the subretinal space in the proper orientation. Although no definite clinical evidence of graft rejection was observed, approaches to enhance long-term survival of grafted tissue may also be necessary. More reliable and reproducible methods of measuring visual function in patients with low levels of vision will also be essential. However, the largest hurdle will likely be the establishment of functional connections between the graft and the host retina. This is certainly an area of active investigation in animal studies of retinal transplantation. Despite these many obstacles, neural retinal transplantation remains one of the few approaches that hold promise for restoring vision to patients with outer neural retinal cell loss.

References

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