Autologous peripheral retinal pigment epithelium translocation in patients with subfoveal neovascular membranes

J C van Meurs, E ter Averst, L J Hofland, P M van Hagen, C M Mooy, G S Baarsma, R W Kuijpers, T Boks, P Stalmans

Aim: To evaluate the possibility of translocating autologous peripheral retinal pigment epithelial (RPE) cells and enhance their adhesion to improve functional outcome after choroidal neovascular membrane extraction in patients with subfoveal neovascular membranes.

Methods: A prospective, non-controlled surgical study in eight consecutive patients operated between February and July 2001 with final data monitoring in July 2002. All patients had mixed subfoveal membranes of 2–4 disc diameters. Functional tests included Snellen vision and central fixation testing. During vitrectomy, after the extraction of the neovascular complex, $8 \times 10^4-16 \times 10^4$ RPE cells were removed from the periphery and translocated under the macula following the submacular injection of 2 $\mu$g of poly-L-lysine to promote adhesion of the cells.

Results: With a follow up ranging from 3 months to 16 months, a pigmented area was seen in the extraction bed of the neovascular membrane in only one patient. Fixation was at the edge of the extraction bed in three patients. Vision remained the same in five patients and deteriorated in three (all with retinal detachment). Retinal detachment due to proliferative vitreoretinopathy occurred in three patients.

Conclusions: The translocation of autologous peripheral RPE cells after membrane extraction was technically possible in a sterile manner, but was associated with a high proliferative vitreoretinopathy rate and in the present series had no measurable positive effect on functional outcome.

Consequently, other modifications to recreate the underlayer under the macula have included the injection of a homologous fetal cell patch. Its lack of success was partly attributed to a chronic host versus graft rejection. Recently, Weichel et al reported the lack of functional improvement after the injection of HLA matched homologous RPE cells.

Therefore, several authors used autologous cells: full thickness en bloc translocation of adjacent macular choroid-choriocapillaris-Bruch’s membrane and RPE cells—translocation by blunt needle aspiration and reinjection of mid-peripheral RPE cells—as well as the translocation of IPE cells. For the last two approaches, however, adherence of the injected cells to the damaged Bruch’s membrane remains uncertain.

We have chosen to translocate peripheral autologous RPE cells. We further tried to enhance RPE cell adherence by the use of poly-L-lysine. Here we report the results of this approach in a case series of eight patients with a subfoveal neovascular membrane.

Patients and methods

Patients with a subfoveal choroidal neovascular membrane that was more than 50% occult on fundus fluorescein angiography (FFA) and larger than 2 disc diameters, with or without submacular blood, were eligible for RPE translocation. Eight patients were included in the study, from 29 January 2001 to 2 July 2001. This study had been approved by the institutional board and written informed consent was obtained from all patients, in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

Preoperative examination included history taking, Snellen vision and a dilated pupil fundus examination, including FFA. Postoperative visits were scheduled at 1, 3, and 6 weeks,
3, 6, 9, and 12 months. The final date of monitoring was 30 July 2002. During each visit Snellen vision testing and a comprehensive examination were performed. At 3, 6, and 12 months fundus pictures were taken and central fixation was tested with the smallest spot (500 μm) of the Haag-Straat slit lamp.

Preoperative measures

Three weeks preoperatively argon green laser coagulation retinopexy was performed to wall off the inferior peripheral retina in preparation of the planned peripheral retinectomy. Three hours before surgery, blood was collected from the patient to obtain autologous serum after clotting.

Surgery

After the induction of a posterior vitreous detachment a complete vitrectomy was performed and a peripheral retinal detachment was created in the walled off inferior retina by the transretinal injection of Ringer’s solution into the subretinal space. The detached retina was removed up to the ora serrata with the vitrectome. If the patient was phakic, lensectomy was performed at this stage.

Harvesting and analysis of RPE cells

We modified a bent 21 gauge aspiration canula by placing a Nylon 8-0 loop on top and connected it to a 10 ml syringe. The RPE cells were gently scraped off Bruch’s membrane and aspirated through the canula. We collected 20 ml Ringer/RPE aspirate. The cell aspirate was injected into a closed sterile 30 ml centrifuge tube and autologous serum (10%) was added to prevent adherence of the cells to the tubing. After centrifuging (3 g, 5 minutes) within a sterile holder the supernatant was aspirated by the surgeon. The cell pellet was centrifuging (3 g, 5 minutes) within a sterile holder the supernatant was aspirated by the surgeon. The cell pellet was harvested and analysis of RPE cells

After 5 minutes RPE cells were injected subfoveally, again using a PFCL bubble to minimise reflux. Video sequences were made during cell injection, to document proper subretinal injection. Either a 15% mixture of C3F8 gas or a silicone oil tamponade was used and additional laser applied at the peripheral retinectomy site. In gas filled eyes, an iris claw fixated IOL was inserted; in silicone filled eyes an IOL was inserted at the second surgery when we removed silicone oil. The patient was instructed to remain supine as much as possible during the first 24 hours.

Postoperative course

Retinal detachment developed due to proliferative vitreoretinopathy (PVR) in three patients, who underwent a second vitrectomy with silicone oil tamponade. Follow up of patients 1 and 3 was only 3 months, because both died. In the other six patients, minimal follow up was 12 months (range 12–16 months). Snellen visual acuity at 3, 6, 9, or 12 months did not show a two line improvement in any patient. Patients 2 and 7 visual loss was clearly caused by the retinal detachment and PVR. These two patients, as well as patient 4, did subjectively worse. Patients 3, 6, and 8 reported some subjective improvement.

DISCUSSION

For patients with large, occult subfoveal membranes with or without subretinal blood present, there is no evidence based treatment available. We have translocated autologous peripheral RPE cells to try and improve both the reported autologous RPE19 and IPE20 injection approaches: the latter by having a formed laser retinopexy at the time of surgery.

RESULTS

Seven patients had ARMD in both eyes, with a fibrovascular scar in the fellow eye in five patients (table 1). One 37 year old woman had a subfoveal membrane secondary to presumed ocular histoplasmosis syndrome (POHS). The preoperative duration of visual loss in the operated eye ranged from 8 weeks to 6 months. Visual acuity of the operated eye ranged from counting fingers (CF) at 1 metre to 6/120.

Preoperative FFAs were obtained in all patients. Membrane size ranged from 2–4 disc diameters. Subretinal blood was present in six patients.

The number of cells analysed per patients varied from 12.5 x 10^3 to 70 x 10^3. Trypan blue exclusion staining revealed that over 90% of cells were viable. The heavily pigmented cells we plated adhered to the dish for 5–28 days. Only the cells of patient 4 grew to confluence, with a hexagonal pattern characteristic for RPE cells.
We aspirated a fairly large number of cells which we showed in a parallel study to be able to survive the aspiration-centrifuge-injection treatment. On cytosin preparation these cells showed RPE specific staining with cytokeratin 8/18 monoclonal antibodies (van Meurs et al, ARVO abstract 2002, no 683). In that study, however, RPE cells from patients with ARMD (although viable as evaluated by trypan blue exclusion) were unable to grow to confluence.

The number of cells we analysed per patient for quality control varied from 12.5 x 10^6 to 70 x 10^6, but this number of analysed cells was only a small portion of the number of cells actually re-injected. As the average size of the denuded area after the extraction of the choroidal membrane was approximately 4 disc areas with an estimated cell number of 7 x 10^6 RPE cells, the transplanted number of RPE cells would be sufficient if not excessive to repopulate the damaged submacular area.

Adhesion of loose clumps of cells to the damaged Bruch’s membrane is important for their survival and is likely to be a problem for the cells we and others have injected. It is not clear how many of the injected cells managed to settle and function metabolically and elaborate substances to re-establish Bruch’s membrane and modulate choriocapillarly growth. We chose to enhance adhesion by the application of a growth. We chose to enhance adhesion by the application of a well known laboratory and graft adhesion promotor poly-L-lysine. We showed in rabbits that poly-L-lysine in the dose used was not retinotoxic (Stalmans et al, 2000). It was uncertain, however, whether the injection of poly-L-lysine had any influence on the adhesion of the injected RPE cells in our patients.

Several factors may have contributed to the high incidence of PVR in this study: (1) The creation of a large retinectomy with aspiration of RPE cells caused a blood-retina barrier breakdown. (2) Our technique to try and prevent reflux through the injection site (sealing with PFCL) was probably not successful: we speculate that at least some RPE cells injected under macula may have flowed back through the injection site into the vitreous cavity and contributed to the development of PVR. (3) The same reflux may have occurred with poly-L-lysine, which may have enhanced the adherence of RPE cells to the retina, thereby accelerating PVR. (4) By selecting an inferior rather than a superior retinectomy site for the transplanted RPE cells under the macula.

The transplantation of autologous cell sheets would decrease the reflux problem and might increase the chance of RPE cells survival, but growth on a sheet and a second operation are challenging in terms of sterility, the choice of the sheet material used, and the surgical placement technique.

Signs of a recurrent choroidal membrane were detected ophthalmoscopically in one patient. As we did not perform fluorescein angiograms to exclude recurrent membranes and our follow up was relatively short (12–18 months), we may be underestimating the rate of recurrence.

Disappointingly, translocated RPE cells were ophthalmoscopically not detectable in seven patients. Several factors may have played a part: reflux to the vitreous cavity preoperatively or postoperatively, because the retinotomy site was not adequately closed and adhesion of the RPE cells to an underlayer never occurred; a rapid clearance of the RPE cells by an inflammatory response secondary to the mechanical removal of the subretinal membrane; loss of pigment due to RPE cell proliferation: non-pigmented RPE cells. Indeed, the pigmentation noted in patient 8 may not be related to the translocated cells, but may merely be a reaction to surgical extraction. Functionally, vision stabilised in five patients; fixation was on the edge of the extraction bed in three patients, but not over the pigmented area in the only patient with visible pigmentation. In three patients their decrease of vision was caused by retinal detachment.

In conclusion, although we were able to translocate RPE cells in a sterile manner, our findings do not support our assumption that the translocated RPE cells would improve on the functional results of simple membrane extraction. Because of the modest results we obtained in the patients without complications, as well as the severe complications we encountered in others, we have stopped this approach until we have learned more about means to promote the survival of transplanted RPE cells under the macula.

### Table 1 Patient characteristics and treatment

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Preop VA</th>
<th>No tested RPE cells</th>
<th>Tamponade</th>
<th>Final VA</th>
<th>Fixation within extraction bed</th>
<th>Follow up (months)</th>
<th>Reoperations</th>
<th>PVR</th>
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<tr>
<td>1</td>
<td>M</td>
<td>88</td>
<td>6/120</td>
<td>15000 C F</td>
<td>6/120</td>
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<td>–</td>
<td>–</td>
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<tr>
<td>2</td>
<td>M</td>
<td>92</td>
<td>6/120</td>
<td>7500 C F</td>
<td>CF</td>
<td>no tested</td>
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<tr>
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<td>76</td>
<td>6/120</td>
<td>40000 SO</td>
<td>6/120</td>
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<td>Better</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
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<td>37</td>
<td>CF</td>
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<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>5</td>
<td>M</td>
<td>76</td>
<td>CF</td>
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<td>6/120</td>
<td>edge</td>
<td>14 Vitrectomy, SO yes</td>
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<td>HM</td>
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<td>Worse</td>
<td>–</td>
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<tr>
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<td>6/120</td>
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<td>edge</td>
<td>12 –</td>
<td>–</td>
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VA = visual acuity, CF = counting fingers, CF3 = counting fingers at 3 metres, HM = hand motions, PVR = proliferative vitreoretinopathy, SO = silicone oil.

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