Histological analysis of retinas sampled during translocation surgery: a comparison with normal and transplantation retinas

L Wickham,1 G P Lewis,2 D G Charteris,1 S K Fisher,2 L Da Cruz1

ABSTRACT

Aims: To carry out a histopathological analysis of retinal specimens of patients undergoing translocation surgery for age-related macular degeneration (ARMD).

Methods: A histopathological analysis, using confocal microscopy, was performed on six retinal specimens. Results were compared with those from two further retinal specimens, collected during RPE transplantation, to control for the effects of vitrectomy and ARMD. In addition, a third control specimen from a cadaver with no history of ophthalmic disease was also analysed.

Results: In the translocation specimens, rods and cones were relatively well preserved but showed reduced density and outer segment length. In four specimens, there were focal areas of rod opsin redistribution to the inner segment, but this was not observed in the controls. Staining with calbindin was decreased in cones compared with controls but normal in horizontal and amacrine cells. Rod bipolar cells were mildly disorganised, and in one there was evidence of neurite sprouting. Glial fibrillar acidic protein was raised in both translocation and transplantation retinae but not in the cadaver control.

Conclusions: In this study, there was little evidence of cellular injury following iatrogenic detachment; however, the rate of PVR following translocation surgery infers that cellular events set in motion may continue despite early reattachment following human retinal detachment have not been well characterised due to the difficulty in obtaining retinal specimens in this group of patients. Data on human detachment derived from retinal specimens obtained during surgery for PVR, in which relieving retinectomies are performed to treat retinal shortening, appear to correlate well with those found in the feline model. Histopathological changes following acute retinal detachment have been well documented in animal models, and in the feline model, retinal detachment followed by rapid reattachment within 1 day resulted in changes in cellular structure, including shortening of outer segments, redistribution of rod and cone opsin and evidence of retinal pigment epithelial cell and Müller cell proliferation.

In this histopathological case series, we present the results of immunohistochemistry on retinal samples obtained from patients undergoing retinal translocation surgery for age-related macular degeneration to determine the effect of 1–2 h of iatrogenic retinal detachment on retinal structure and thus increase our understanding of events leading to the development of PVR in the postoperative period.

MATERIALS AND METHODS

This study was approved by the local Research Ethics Committee, and written informed consent was obtained from each participant. Retinal specimens were obtained from six patients at the time of translocation surgery (table 1). Two further retinal specimens, collected at the time of retinal pigment epithelial transplantation, were used to control for the effects of vitrectomy and ARMD on retinal histopathology and are henceforth referred to as transplantation controls. In addition, a third control specimen was also obtained from a 75-year-old male cadaver with a diagnosis of prostate cancer to provide an age-matched control without age-related macular degeneration or history of vitreoretinal surgery. The eye was retrieved within 7 h of death and placed in paraformaldehyde.

Collection of retinal specimens

Retinal translocation

Retinal translocation surgery was performed as previously described. In brief, a three-port pars plana vitrectomy was performed. Retinal detachment was achieved by injecting balanced salt solution (BSS) into the subretinal space using a 42-gauge subretinal cannula. Following complete retinal detachment, a 360° retinotomy was performed...
The neurosensory retina was then gently peeled off the surface along the ora serrata. Retinal samples were then taken from the edge of the retinotomy. At the time of sampling, the retina had been detached for 1–2 h. Following excision, retinal specimens were immediately fixed in 4% paraformaldehyde in sodium cacodylate buffer (0.1 M; pH 7.4; Electron Microscopy Sciences, Fort Washington, Pennsylvania) for a minimum of 24 h. After rinsing in PBS, the specimens were embedded in 5% agarose (Sigma, St Louis, Missouri) in PBS. Sections (75 μm thick) were cut using a vibratome (Technical Products International, Polysciences, Warrington, Pennsylvania) and incubated in normal donkey serum (1:20; Jackson ImmunoResearch, West Grove, Pennsylvania) in PBTA, that is PBS containing 0.5% bovine serum albumin (BSA; Fisher Scientific, Pittsburgh, Pennsylvania), 0.1% Triton X-100 (Boehringer Mannheim, Indianapolis, Indiana) and 0.1% sodium azide (Sigma, St Louis, Missouri) overnight at 4°C on a rotator. After removal of blocking serum, primary antibodies were added (table 2).

### Immunohistochemistry

After rinsing in PBS, the specimens were embedded in 5% agarose (Sigma, St Louis, Missouri) in PBS. Sections (75 μm thick) were cut using a vibratome (Technical Products International, Polysciences, Warrington, Pennsylvania) and incubated in normal donkey serum (1:20; Jackson ImmunoResearch, West Grove, Pennsylvania) in PBTA, that is PBS containing 0.5% bovine serum albumin (BSA; Fisher Scientific, Pittsburgh, Pennsylvania), 0.1% Triton X-100 (Boehringer Mannheim, Indianapolis, Indiana) and 0.1% sodium azide (Sigma, St Louis, Missouri) overnight at 4°C on a rotator. After removal of blocking serum, primary antibodies were added (table 2).

### Table 2 Antibodies studied

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Manufacturer</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Calbindin</td>
<td>Sigma (St Louis, Missouri)</td>
<td>1:1000</td>
</tr>
<tr>
<td>GFAP</td>
<td>Dako (Carpinteria, California)</td>
<td>1:400</td>
</tr>
<tr>
<td>Neurofilm</td>
<td>Biomedia Corp (Foster City, California)</td>
<td>1:500</td>
</tr>
<tr>
<td>Synaptobrevin</td>
<td>Synaptic Systems (Göttingen, Germany)</td>
<td>1:500</td>
</tr>
<tr>
<td>M-cone opsin</td>
<td>Chemicon Int (Temenes, California)</td>
<td>1:500</td>
</tr>
<tr>
<td>PKC</td>
<td>Biomol research labs (Plymouth Meeting, Pennsylvania)</td>
<td>1:100</td>
</tr>
<tr>
<td>Rhodopsin</td>
<td>Santa Cruz Biotechnology (Santa Cruz, California)</td>
<td>1:100</td>
</tr>
<tr>
<td>PNA-biotin</td>
<td>Vector laboratories (Burlingame, California)</td>
<td>1:1000</td>
</tr>
</tbody>
</table>

GFAP, glial fibrillary acidic protein; PKC, protein kinase C; PNA-biotin, biotinylated peanut agglutinin.

### RESULTS

Similar changes in protein expression were observed in five of the six translocation specimens analysed; however in one sample (patient 5) more advanced pathology was seen. This patient was not known to have any previous retinal disease or surgery before undergoing retinal translocation.

Results of confocal microscopy are displayed in table 3. Calbindin labelling of cones and cells in the inner nuclear layer (INL) (horizontal and amacrine cells) was performed in four of the six translocation samples. Staining of the cones was reduced in all of the four specimens tested.

Analysis of second-order neurons showed mild disorganisation of the rod bipolar cells in all translocation specimens (labelled with protein kinase C (PKC), red). In one patient (patient 5) there were more marked changes with some neurite sprouting into the outer nuclear and plexiform layers (fig 2F, arrow). Antisynaptobrevin was used to label photoreceptor terminals in the outer plexiform layer (OPL). In four patients, there were gaps in the OPL associated with areas of rod opsin redistribution (fig 2G, green). In one patient (patient 5) gaps in the OPL were associated with clusters of cone pedicles and spherules (fig 2G, green). On labelling for neurofilament, a low-intensity stain was observed within the horizontal cells in all of the specimens analysed including the controls (fig 2A–C, green).

Staining for glial fibrillary acidic protein (GFAP) was increased in all translocation samples (fig 2I, green) and the transplantation controls (fig 2H, green) but not the cadaver control (fig 2G, green). In the cadaver control, anti-GFAP labelled the intermediate filaments in Müller cell endfeet and astrocyte processes in the ganglion cell layer (fig 2G), whereas in all translocation samples and transplantation controls, GFAP labelling extended from the internal limiting membrane (ILM) to the outer limiting membrane (OLM). In one specimen (patient 5), a Müller cell was seen just beginning to breach the OLM (fig 2I, arrow).

After overnight incubation at 4°C on a rotator, sections were rinsed in PBTA and incubated again overnight at 4°C with the secondary antibody. Donkey antimouse and donkey antirabbit secondary antibodies were used for each combination of primary antibodies, conjugated to Cy2 or Cy3 (Jackson ImmunoResearch). All secondary antibodies were used at a dilution of 1:200, and all the antibodies were diluted in PBTA. The sections were then rinsed, mounted in N-propyl gallate in glycerol and viewed on a laser scanning confocal microscope (Fluoview 500; Olympus, Tokyo).

### DISCUSSION

In this study, samples taken 1–2 h following induction of a retinal detachment (just prior to retinal reattachment) showed only minor changes in cellular structure compared with that seen in transplantation controls. Although this study was conducted on a small number of specimens with the limitations that this entails, our findings show similarities to those observed in the feline model, where the effects of retinal detachment have been studied more extensively. Changes in the distribution of protein expression are likely to require more than 1–2 h, and so many of the findings described in both the translocation and transplantation specimens may not have occurred in the time course of the operation but probably reflect the underlying disease process.

It should also be noted that the area of retina sampled in the translocation and transplantation specimens differed slightly. In the translocation specimens, retina was sampled anterior to the
Figure 1  Double-label laser scanning confocal images showing the distribution of rod opsin (red) and synaptobrevin (green) in the cadaver control (A) and a translocation specimen (B1, B2). (A) shows normal distribution of rod opsin and synaptobrevin in the cadaver control. In the translocation sample (B1, B2) rod outer segments (OS) were decreased in density and length. Antisyntaptobrevin labels the photoreceptor terminals. In four out of six translocation specimens, gaps in the outer plexiform layer (OPL) were associated with areas of rod opsin redistribution (B1 arrow) and clusters of cone pedicels and spherules (B1 (arrowhead) and B2 (high magnification, arrow).) Magnification denoted by scale bar in A and C. GCL, ganglion cell layer; INL, inner nuclear layer; IPL, inner plexiform layer; IS, inner segment; ONL, outer nuclear layer.

Figure 2  Double-label laser scanning confocal images showing changes in the distribution of several proteins. M cone opsin (A, B, C, red) showed a reduction in outer segment (OS) length in the translocation specimen (C) compared with the cadaver (A) and the transplantation control specimens (B). In the cadaver control there was some redistribution of cone opsin to the inner segment (A, arrow). Low-intensity neurofilament staining is seen in the horizontal cells (A, C, green). Calbindin staining of cones is decreased in the translocation samples (F, green) compared with that in the transplantation (E) and cadaver (D). Calbindin labelling of cells in the inner nuclear layer (INL) was normal in all samples (D–F). Protein kinase C (D–F, red) labels bipolar cells and was normal in the cadaver and transplantation samples. In the translocation specimen, there was mild disorganisation of the rod bipolar cells with neurite sprouting into the outer nuclear layer (ONL) and outer plexiform layer (OPL) (F, arrow). The interphotoreceptor matrix associated with cones was labelled with biotinylated peanut agglutinin (PNA) (G–I, red) and showed a reduction in cone density in translocation (I) and transplantation (H) specimens compared with the cadaver (G). Staining for glial fibrillar acidic protein (GFAP) (G–I, green) was increased in the translocation (I) and transplantation (H) specimens but not the cadaver control (G). Magnification is depicted in figure G; where magnification differs, a yellow scale bar denotes 50 \( \mu \text{m} \). GCL, ganglion cell layer; IPL, inner plexiform layer; IS, inner segment; PKC, protein kinase C.
equator compared with transplantation specimens which were taken near the equator. This is unlikely to have had an effect on the structural changes observed between the two groups but it may explain the observed differences in retinal thickness. The apparent shortening of photoreceptor outer segments and isolated areas of rod and cone opsin redistribution observed in the translocation and transplantation specimens have also been described in photoreceptors overlying drusen in patients with age-related macular degeneration.13 This could explain the focal areas of opsin redistribution but is unlikely to account for the widespread reduction in outer segment density and length observed in the retinal detachment specimens, and this may reflect damage caused by separating the neural retina from the RPE. Rod opsin redistribution was also noted in the cadaver control, but in this lab we have observed similar changes in cats as a result of fixation delay postmortem (CP Lewis, personal communication).

Increased anti-GFAP labelling was seen in all of the specimens, including the AMD transplantation control, but was not observed in the cadaver eye. Although Müller cell hypertrophy has been observed within 2 days of retinal detachment, the extent of GFAP labelling observed in our samples may not have occurred within the 2 h between onset of retinal detachment and retinal sampling.15 GFAP expression is a non-specific indicator of stress and has been reported to occur in response to a number of retinal pathologies including age-related macular degeneration.20–22 An increase in GFAP immunolabelling has also been demonstrated in aged normal retinas compared with young controls, and it is therefore likely that increases in GFAP observed in this study are a reflection of the underlying disease process or perhaps an age-related phenomenon.23–24 There did not appear to be any association between the intensity of GFAP staining and the final anatomical outcome of surgery or the development of PVR, but the number of samples examined is small.

In our study, anti-calbindin D, which labels cones in their entirety, appeared to be greatly reduced in all samples compared with the control. Similarly, in detached feline retina, calbindin labelling of the cones is greatly reduced following 3 days of detachment, but once reattached, labelling of the cone cell cytoplasm shows an intensity similar to normal.24 Recovery of photoreceptors following retinal reattachment has been demonstrated, although outer segment length and photoreceptor cell numbers are still significantly reduced compared with normal controls.25

Analysis of second-order neurons showed mild disorganisation of the rod bipolar cells with some gaps in the OPL. Antisynaptobrevin was used to demonstrate the organisation of the photoreceptor terminals in the OPL and appeared to be normal in all but one patient. In this patient (patient 5) there were gaps in the OPL associated with clusters of cone pedicles and spherules (fig 1B1). A similar observation has also been reported in PVR, where advancing photoreceptor loss was accompanied by thinning of the OPL and surviving rod terminals clustering around cone pedicles.5 This patient, however, had not undergone previous surgery and did not develop PVR following transplantation surgery.

The findings in this study support those reported in animal studies showing that retinal reattachment within 1 h or 1 day is very effective at preventing or reversing the cellular changes of retinal detachment, but that cellular proliferation may persist for longer.5 In this study, some of the proteins examined showed minor alterations in expression suggesting that a response to retinal detachment is being initiated. Events preceding changes in gene expression and cellular morphology following retinal detachment have been studied in cat and rabbit retinas using early transcription factors.18 A rapid response to retinal detachment has been shown to occur within 15 min, including phosphorylation of fibroblast growth factor receptor (FGFR-1) and increased expression by RPE and Müller cells of extracellular signal-regulated kinase (ERK) and activator protein (AP-1) transcription factors.19 In vivo experiments in rabbits and cats have also shown that an intravitreal injection of 1 μg of bFGF leads to FGF receptor internalisation, Müller cell proliferation and increased expression of GFAP and vimentin.24 It is possible therefore that in some patients, the cellular events initiated by this rapid response to retinal detachment may persist despite reattachment resulting in the development of PVR following translocation surgery.

In conclusion, at the time of retinal reattachment there appeared to be little evidence of cellular injury as a result of surgery, but the rate of PVR following translocation surgery infers that retinal reattachment may not be sufficient to reverse cellular events set in motion by detachment. Early transcription factors were not investigated in this study but would provide additional information on the processes initiated by iatrogenic retinal detachment in translocation surgery.

Competing interests: None.

Ethics approval: Ethics approval was provided by Moorfields Eye Hospital and the Whittington Hospital.

Patient consent: Obtained.

REFERENCES
Histological analysis of retinas sampled during translocation surgery: a comparison with normal and transplantation retinas

L Wickham, G P Lewis, D G Charteris, et al.

Br J Ophthalmol 2009 93: 969-973 originally published online December 17, 2008
doi: 10.1136/bjo.2008.146613