Silicone oil concentrates fibrogenic growth factors in the retro-oil fluid

R H Y Asaria, C H Kon, C Bunce, C S Sethi, G A Limb, P T Khaw, G W Aylward, D G Charteris

Aim: To determine whether silicone oil concentrates protein and growth factors in the retro-oil fluid.

Methods: A laboratory analysis of intraocular fluid and vitreous specimens obtained from patients undergoing removal of silicone oil, revision vitrectomy, or primary vitrectomy for macular hole, proliferative vitreoretinopathy (PVR), or retinal detachment. Patients were prospectively recruited from routine vitreoretinal operating lists. Vitreous cavity fluid and vitreous samples were analysed for the presence of transforming growth factor beta (TGF-β2), basic fibroblast growth factor (bFGF), interleukin 6 (IL-6), and total protein using either commercially available enzyme linked immunosorbent assays (ELISA) or protein assay kits.

Results: The median levels of bFGF, IL-6, and protein in the retro-oil fluid were raised (p<0.05) compared to all the other vitreous and vitreous cavity fluid samples. bFGF, IL-6, and protein levels were raised in PVR vitreous compared to non-PVR vitreous. TGF-β2 levels were not significantly raised in retro-oil fluid or in PVR vitreous.

Conclusions: The concentration of fibrogenic (bFGF) and inflammatory (IL-6) growth factors and protein is raised in retro-silicone oil fluid. This may contribute to the process of retro-oil perisilicone proliferation and subsequent fibrocellular membrane formation.
undiluted vitreous or vitreous cavity fluid samples were collected by aspiration through the vitreous cut. Undiluted samples were divided into aliquots in siliconised tubes (Eppendorf, Freemont, CA, USA) and kept frozen at −70°C until each analysis.

**Enzyme linked immunosorbent assays (ELISA)**

Levels of TGF-β2, bFGF, and IL-6 were analysed using sandwich enzyme immunoassay kits (B&D Systems, Oxon, UK). Pilot studies were performed to determine the appropriate dilutions for each growth factor. The sample volumes used were 200 μl (1:24 dilution) for TGF-β2, 150 μl (1:15 dilution) for bFGF, and 200 μl (1:75 dilution) for IL-6. The minimum detectable concentrations (sensitivity) for the assay kits were 2.00 pg/ml, 0.043 pg/ml, and 0.08 pg/ml for TGF-β2, bFGF and IL-6 respectively. Since the assay for TGF-β2 only detects its active form, samples to be analysed for this factor were first activated by the addition of 1 M HCl (40 μl HCl/ 200 μl sample). These were then neutralised with 40 μl 1.2 M NaOH/ 0.5 M HEPES. The assays therefore measured the total amount of potentially active TGF-β2.

**Protein analysis**

The total protein concentrations of the samples were measured using a commercial assay (Protein Microassay; BioRad, Herts, UK). This colorimetric assay is a solution of cupric ions that forms a copper-protein complex (coloured compound) with protein. It allows rapid screening of multiple small volume fluid and vitreous samples.

**Statistical analysis**

Growth factor levels were compared between retro-oil fluid and all other vitreous and vitreous fluid samples individually using the Wilcoxon rank sum (Mann-Whitney) test. The retro-oil fluid samples were also compared with the control specimens combined. Selected comparison of control specimens was carried out where these were considered to be biologically relevant: revision vitrectomy fluid was compared to PVR and to non-PVR vitreous and PVR vitreous was compared to non-PVR vitreous. Non-parametric methods were used because the data showed considerable skewness. No adjustment was made for multiple testing because the analysis was hypothesis generated. Statistical calculations were performed using commercial software (Stata, StataCorp. 2003. Stata Statistical Software: Release 8.0. College Station, TX, USA: StataCorp LP).

**RESULTS**

Complete data were available for all patients. Of the 13 patients undergoing removal of silicone oil from whom retro-oil fluid was obtained two had had previous (preoperative) PVR and seven had evidence of new proliferation (postoperative) PVR before oil removal. In these patients the duration of silicone oil tamponade ranged from 1 to 26 months with a median of 3 months. Two patients had oil in situ for longer than 6 months but their protein and growth factor levels were not markedly different from those with lesser durations. All 13 retro-oil fluid patients had had silicone oil injected because of clinical features suggestive of a high risk of postoperative PVR development: two had existing PVR, two had had previous retinal detachment surgery, one had a giant retinal tear, and the others had large or multiple retinal breaks.

In the revision vitrectomy fluid group three of 11 cases had PVR: 34 of the total of 298 non-retro-oil control samples had PVR.

The median levels of growth factors and protein for the various groups are documented in table 1. The probability values for the statistical analyses of comparisons of medians using the Mann-Whitney test are presented in table 2. The median levels of bFGF and IL-6 in the retro-oil fluid were significantly raised compared to all other vitreous and vitreous fluid samples except vitreous from eyes with PVR (table 2) and the IL-6 value in revision vitrectomy. Protein in retro-oil fluid was raised in comparison with all other specimens. There was little evidence of any difference, however, between the median level of TGF-β2 in the retro-oil fluid compared to the other samples (tables 1 and 2).

PVR vitreous had raised levels of bFGF, IL-6, and protein but not TGF-β2 when compared to non-PVR vitreous (tables 1 and 2). Levels of growth factors and protein in revision vitrectomy fluid showed little difference from vitreous from eyes with or without PVR apart from a significantly raised level of bFGF in eyes with PVR (tables 1 and 2).

<table>
<thead>
<tr>
<th>Table 1 Patient groups, growth factor, and protein levels</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Sample type</strong></td>
</tr>
<tr>
<td>-----------------</td>
</tr>
<tr>
<td>Retro-oil fluid (n = 13)</td>
</tr>
<tr>
<td>Macular hole vitreous (n = 10)</td>
</tr>
<tr>
<td>Revision-vitrectomy fluid (n = 11)</td>
</tr>
<tr>
<td>No preoperative PVR vitreous (n = 244)</td>
</tr>
<tr>
<td>Preoperative PVR vitreous (n = 31)</td>
</tr>
<tr>
<td>Non retro-oil samples combined</td>
</tr>
</tbody>
</table>

| IQR, interquartile range. |
samples obtained from patients with PVR. The consistency of the results of growth factor and protein analyses between control specimens (and for the individual growth factors, see tables 1 and 2) is further evidence that the controls used are valid.

An effect of the underlying pathology on growth factor levels cannot be discounted, again the use of multiple controls helps to define the role of silicone oil in the altered growth factor concentrations we have demonstrated. It is notable that levels of growth factors were not significantly different in retro-oil fluid from PVR vitreous suggesting that the presence of PVR (seen in seven of 13 retro-oil fluid specimens) has an important influence on growth factor pathobiology.

Basic FGF has been shown to enhance the proliferation of Muller cells, retinal astrocytes, and retinal pigment epithelial cells in vivo, and has previously been shown to be elevated in the vitreous of eyes developing PVR (a similar finding to that of the analysis of controls in this study) and clearly has the potential to have a role in mediating the proliferative and fibrogenic responses in PVR. Raised levels in the intraocular fluid surrounding silicone oil point to a potential similar role in perisilicone proliferation.

IL-6 is an important mediator of the acute phase reaction in inflammatory and immune responses. Cells with IL-6 mRNA expression have been found in PVR epiretinal membranes and previous work has also have shown that the vitreous levels of IL-6 are significantly raised in vitreous samples obtained from patients with PVR. The consistent finding of raised IL-6 levels is evidence for its role in the marked blood-retinal barrier breakdown seen in PVR.

Both basic FGF and IL-6 have the potential to contribute to the formation of fibrocellular epiretinal membranes and the concentration of these growth factors found in the retro-silicone oil fluid points to a possible pathogenic mechanism for perisilicone membrane formation. It is notable that animal studies have demonstrated silicone oil can have an enhanced mitogenic effect on RPE cells and an increased PVR rate in silicone filled vitreous.

The present investigation has additionally shown that there is an increase in total protein in the retro-oil fluid. This suggests that the increased levels of bFGF and IL-6 may be the result of enhanced active secretion rather than a more generalised concentration effect of all soluble mediators. Alternatively there may be an enhanced tissue uptake and/or clearance of TGF-β and it may still have an important biological effect in PVR related fibrogenesis. An additional possibility is that, since our methodology detects total TGF-β, there may be an increase in biologically active growth factor in the eye which we have not detected and which could enhance PVR development.

The nature and incidence of perisilicone proliferation remain uncertain. Previous reports have documented recurrent epiretinal proliferation behind silicone oil. Lewis et al found that 19 of 31 eyes (61%) developed perisilicone proliferation (and that this led to redetachment in 15 eyes) and Zilis et al reported 21 of 55 eyes (38%) developed perisilicone proliferation. In the silicone study, however, there was no difference in prevalence of postoperative macular pucker between eyes randomised to gas or to silicone oil, suggesting that both may have an effect on postoperative epiretinal membrane formation. The purity of the silicone oil used may have an important role in the incidence of secondary proliferation in these eyes and it has been shown experimentally that contaminated silicone oil has an enhanced effect on retinal pigment epithelium proliferation compared to purified, medical grade silicone oil. Improvements in the quality of intraocular silicone oils since their initial introduction can therefore be anticipated to result in a decline in the incidence of perisilicone proliferation.

Oil purity, however, may not be the only factor leading to a predisposition to secondary membrane formation and the evidence of this study is that, although purified, medical grade silicone oil was used in all cases there remains a concentration effect on potentially fibrogenic mediators. Despite these caveats silicone oil remains a vital tool in the management of complex retinal detachment. Vitreoretinal surgeons, however, should be aware that its biological effects will alter intraocular physiology and can potentially contribute to enhanced fibrous scarring. In addition, the presence of silicone oil will alter the pharmacodynamics of any adjunctive treatments aimed at modifying the PVR process potentially producing a concentrated “depot” in the retro-oil fluid.

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REFERENCES

Table 2 Probability (p) values for comparisons of specimens (Wilcoxon rank sum [Mann-Whitney] test)

<table>
<thead>
<tr>
<th>Comparison</th>
<th>TGF-β</th>
<th>bFGF</th>
<th>IL-6</th>
<th>Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td>Retro-oil v macular hole</td>
<td>0.24</td>
<td>0.002</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>Retro-oil v revision vitrectomy</td>
<td>0.54</td>
<td>0.0007</td>
<td>0.32</td>
<td>0.005</td>
</tr>
<tr>
<td>Retro-oil v non-PVR vitreous</td>
<td>0.76</td>
<td>0.0001</td>
<td>0.0003</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Retro-oil v PVR vitreous</td>
<td>0.72</td>
<td>0.063</td>
<td>0.032</td>
<td>0.015</td>
</tr>
<tr>
<td>PVR vitreous v non-PVR vitreous</td>
<td>0.27</td>
<td>&lt;0.0004</td>
<td>0.035</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>Revision vitrectomy v non-PVR vitreous</td>
<td>0.86</td>
<td>0.69</td>
<td>0.086</td>
<td>0.17</td>
</tr>
<tr>
<td>Revision vitrectomy v PVR vitreous</td>
<td>0.29</td>
<td>0.0027</td>
<td>0.92</td>
<td>0.18</td>
</tr>
<tr>
<td>Retro-oil v combined control specimens</td>
<td>0.75</td>
<td>0.0002</td>
<td>0.0005</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>


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