Clinicopathological correlation of epiretinal membranes and posterior lens opacification following perfluorohexyloctane tamponade

Paul Hiscott, Raymond M Magee, Matthew Colthurst, Noemi Lois, David Wong

Abstract

Background/aims—Epiretinal and retrolental proliferation may occur during prolonged use of the novel tamponade agent perfluorohexyloctane (F₆H₈). This study aims to determine whether there is any histological evidence that F₆H₈ has a role in the formation of these membranes.

Methods—Eight epiretinal membranes and three opaque posterior lens capsules were excised from patients in whom F₆H₈ had been used as a long term retinal tamponade agent. The membranes and capsules were examined employing light microscopic methods, including immunohistochemistry.

Results—The epiretinal membranes showed histological features typical of proliferative vitreoretinopathy (PVR), but they also exhibited a dense macrophagic infiltration. In addition, three of the membranes contained multinucleated cells. Macrophages represented up to 30% of the cells present and appeared to contain large intracytoplasmic vacuoles. Similar cells were seen on the back of the posterior lens capsule in one specimen and all three capsules had posterior migration of lens epithelium.

Conclusion—The pathological findings are not simply those of PVR. The macrophage infiltration suggests that there may be a biological reaction to F₆H₈ which could reflect its surmised propensity to emulsify. Further investigations concerning the cellular response to this promising tamponade agent are warranted.

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Material and methods

Silicone oil and long acting gases have been shown to be effective as internal tamponade agents for the management of difficult rhegmatogenous retinal detachments (RRD), especially those complicated by proliferative vitreoretinopathy (PVR). Both gas and silicone have a lower specific gravity than vitreous fluid and, when used as internal tamponade, they occupy the upper part of the vitreous cavity providing good support for the superior retina. However, PVR has a propensity for the inferior retina and the tamponade effect of gas and silicone oil may then be deficient when the fill is subtotal. Thus, there may be a specific role for a tamponade agent with a higher specific gravity than vitreous.

The semifluorinated alkanes (SFA) are a group of novel substances which have the potential to act as internal tamponade agents for the inferior fundus (reviewed by Colthurst et al). They are immiscible with aqueous and their interfacial tensions are comparable with the perfluorocarbon liquids (PFCL) and with silicone oil. At 1.3–1.35 g/cm³, SFA have a higher specific gravity than vitreous. Moreover, SFA have a lower specific gravity than PFCL and theoretically may cause less trophic effect on the retina than PFCL. Indeed, in a rabbit investigation the SFA perfluorohexyloctane (F₆H₈) was left in the vitreous cavity for up to 3 months and appeared to be well tolerated. As a result of this investigation, F₆H₈ has been used clinically as a long term vitreous tamponade and the results of a multicentre pilot study on the use of F₆H₈ in a series of complicated retinal detachments have been presented recently.

Some of the patients in the F₆H₈ pilot study developed recurrent retinal detachment with widespread PVR. Membranous proliferation was also observed on the posterior surface of the crystalline lens in a few phakic patients. Clinically, it was not possible to determine whether the epiretinal and retrolental membranes were the result (or an exacerbation) of the original disease process or a pathobiological response to the tamponade agent. To help resolve this uncertainty, we studied the histology of excised epiretinal membranes and posterior lens opacifications that had developed during prolonged F₆H₈ tamponade.

Eight epiretinal membranes and three posterior lens opacifications were obtained from eight patients during closed pars plana microsurgery for PVR which had (re)occurred during F₆H₈ tamponade (Table 1). Five of the patients were female and three were male. The age range was 29–87 years. The initial indication for retinal tamponade was PVR in three patients, foveal relocation (two patients), and penetrating/blunt trauma with 360° iris dialysis, total retinal detachment, and expulsive haemorrhage (one patient each). F₆H₈ was the only tamponade employed in five of the eyes. In the remaining three patients, another tamponade agent had been present in the eye before F₆H₈ instillation (Table 1). The duration of F₆H₈ fill varied from 4 to 21 weeks. PVR was the indication for reoperation in all eight patients.
Table 1  Clinical details of the eight patients with F6H8 tamponade

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Age</th>
<th>Sex</th>
<th>Aetiology of retinal disease</th>
<th>Initial retinal status</th>
<th>Tamponade agent</th>
<th>Duration of F6H8 fill</th>
<th>Retinal status on F6H8 removal</th>
<th>Specimens</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54</td>
<td>Male</td>
<td>RRD</td>
<td>PVR grade CP4</td>
<td>F6H8 only</td>
<td>8 weeks</td>
<td>PVR grade CP†</td>
<td>ERM</td>
</tr>
<tr>
<td>2</td>
<td>87</td>
<td>Female</td>
<td>Pseudophakic RD</td>
<td>PVR grade CP4</td>
<td>F6H8 only</td>
<td>10 weeks</td>
<td>PVR grade CP†</td>
<td>ERM</td>
</tr>
<tr>
<td>3</td>
<td>29</td>
<td>Male</td>
<td>B/P trauma</td>
<td>360° iris dialysis</td>
<td>F6H8 only</td>
<td>9 weeks</td>
<td>PVR grade CP</td>
<td>ERM</td>
</tr>
<tr>
<td>4</td>
<td>65</td>
<td>Male</td>
<td>Expansive haemorrhage</td>
<td><em>Total detached</em></td>
<td>SO 2 days, then F6H8</td>
<td>10 weeks</td>
<td>PVR grade CP12</td>
<td>ERM</td>
</tr>
<tr>
<td>5</td>
<td>87</td>
<td>Female</td>
<td>Foveal relocation</td>
<td>180° retinotomy</td>
<td>F6H8 only</td>
<td>7 weeks</td>
<td>PVR grade CP6</td>
<td>ERM+PLC</td>
</tr>
<tr>
<td>6</td>
<td>81</td>
<td>Female</td>
<td>Foveal relocation</td>
<td>180° retinotomy</td>
<td>F6H8 only</td>
<td>9 weeks</td>
<td>PVR grade CP6</td>
<td>ERM+PLC</td>
</tr>
<tr>
<td>7</td>
<td>32</td>
<td>Female</td>
<td>RRD</td>
<td>Total detached</td>
<td>SF6 1 week, then F6H8</td>
<td>4 weeks</td>
<td>PVR grade CP4</td>
<td>ERM</td>
</tr>
<tr>
<td>8</td>
<td>59</td>
<td>Male</td>
<td>MM local resection</td>
<td>PVR grade CP8</td>
<td></td>
<td>21 weeks</td>
<td>PVR grade CP†</td>
<td>ERM+PLC</td>
</tr>
</tbody>
</table>

PVR = proliferative vitreoretinopathy (grading according to the system of Machemer et al.); RRD = rhegmatogenous retinal detachment; ERM = epiretinal membrane; PLC = posterior lens capsule; B/P = blunt/penetrating; MM = malignant melanoma (choroidal); SO = silicone oil; SF6 = sulphur hexafluoride. †No retinal inner limiting lamina contained in specimen.

Table 2  Vacuolated macrophages in F6H8 related PVR epiretinal membranes

<table>
<thead>
<tr>
<th>ERM from patient number</th>
<th>Distribution of vacuolated macrophages</th>
<th>Proportion of vacuolated macrophages as % of total cells</th>
<th>Multinucleated giant cells (% of total cells)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Isolated cells, foci and layers</td>
<td>10</td>
<td>+ (5%)</td>
</tr>
<tr>
<td>2</td>
<td>Isolated cells, foci</td>
<td>10</td>
<td>+ (5%)</td>
</tr>
<tr>
<td>3†</td>
<td>Isolated cells, foci and layers</td>
<td>30</td>
<td>+ (5%)</td>
</tr>
<tr>
<td>4†</td>
<td>Isolated cells, foci</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Isolated cells, foci and layers</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>Isolated cells, layers</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>7†</td>
<td>Isolated cells, foci and layers</td>
<td>20</td>
<td>–</td>
</tr>
<tr>
<td>8</td>
<td>Isolated cells, layers</td>
<td>30</td>
<td>–</td>
</tr>
</tbody>
</table>

ERM = epiretinal membrane.  
*Specimen contained capillaries.  
†No retinal inner limiting lamina contained in specimen.  
+ present; – absent.

Results  
EPIRETINAL MEMBRANES  
All eight epiretinal membranes were fibrocellular in nature. Six were avascular and two contained capillaries (Table 2, Fig 1). Fragments of retinal inner limiting lamina were present in six membranes (Table 2, Fig 1). Various amounts of intracellular and extracellular pigment was observed in all eight specimens (Figs 1 and 2). All the membranes contained glial and RPE cells, which were arranged in foci, layers, and/or distributed as isolated cells in the tissue (Fig 1). The collagenous tissue contained fibroblastic cells and some of these cells were immunoreactive for cytokeratins (Fig 2). Scattered lymphocytes were present in six membranes and occasional plasma cells were observed in one. Neutrophils were not found in any specimen.

All of the membranes contained CD68 positive, cytokeratin negative macrophages (Table 2, Figs 1 and 2). These cells exhibited large intracytoplasmic vacuoles which tended to compress the cytoplasm to a thin peripheral rim and displace the nucleus to the cell periphery (Fig 1). The vacuolated macrophages represented between 5% and 30% of the total cell population of the membranes (Table 2). They were inclined to form clusters or layers in the membranes, although each membrane also contained isolated vacuolated macrophages scattered through the tissue (Table 2, Fig 1). In addition to the vacuolated macrophages, three of the epiretinal membranes contained multinucleated giant cells (Table 2, Figs 1 and 2), many of which contained vacuoles similar to those seen in the macrophages. The multinucleated cells also showed CD68 positivity and represented a relatively small proportion (5%) of the total cell population in each membrane (Table 2, Fig 2).

POSTERIOR LENS CAPSULES  
All three of the samples consisted of segments of lens capsule with lens epithelium on one surface (Table 3). The epithelium formed a monolayer of cuboidal cells in two of the specimens. In the third, the epithelial cells appeared as a thin layer of fibroblastic cells (Table 3, Fig 2). In this third specimen, there also was a pigmented, cellular “retrolental membrane” on the posterior surface of the capsular material (Table 3, Fig 2). This membrane consisted of layers of vacuolated macrophages, similar to those seen in the epiretinal membranes, together with cytokeratin positive fibroblastic...
cells (Fig 2). Glia, lymphocytes, plasma cells, neutrophils, and giant cells were not seen in this specimen.

Discussion

Our results show that PVR membranes forming in the presence of F6H8 have many of the architectural features of PVR membranes evolving in the absence of a tamponade agent. Thus the membranes examined in this study were fibrocellular and, apart from two membranes with a few capillaries, avascular. Furthermore, the majority of the specimens contained fragments of inner limiting lamina from the retina. Moreover, as also is typical for PVR, the epiretinal membranes contained a mixed cell population. This population comprised glia, RPE, fibroblasts (including fibroblastic cells of RPE origin—that is, cytokeratin positive fibroblast like cells), and inflammatory cells.

Characteristically, inflammatory cells are not prominent components of PVR epiretinal membranes. Hence, small numbers of macrophages can be found in about a third of PVR epiretinal membranes arising in the absence of tamponade.
a tamponade agent, although epiretinal membranes of other aetiological types may contain a more prominent macrophage component. In contrast, all of the PVR membranes evolving in the presence of F6H8 contained abundant macrophages and in two of these specimens macrophages represented about 30% of the cells. These cells expressed CD68 and were cytokeratin negative, indicating that they were macrophages rather than RPE cells which had “transdifferentiated” to a macrophage-like phenotype.

Immunohistochemical studies show that macrophage-like RPE are occasionally ob-

Table 3  Histopathological features of the three posterior lens capsule opacifications arising in the presence of F6H8

<table>
<thead>
<tr>
<th>Patient number</th>
<th>Posterior migration of lens epithelial cells</th>
<th>Fibroblastic metaplasia of lens epithelial cells</th>
<th>Retrolental membrane</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>6</td>
<td>+</td>
<td>−</td>
<td>−</td>
</tr>
<tr>
<td>8</td>
<td>+</td>
<td>+</td>
<td>+*</td>
</tr>
</tbody>
</table>

+ present; − absent.
* The retrolental membrane comprised vacuolated macrophages and cytokeratin positive fibroblastic cells (Fig 2).

![Figure 2](http://example.com/figure2.png)
served as a major component of retrosilicone oil PVR membranes (that is, membranes forming behind a silicone oil tamponade) and, in this situation, appear distended by spherical, presumed silicone oil-containing intracytoplasmic vacuoles. However, similar studies on larger numbers of membranes indicate that macrophages (as opposed to cells of RPE origin) may predominate in most retrosilicone oil membranes. Moreover, it is thought that, in these membranes, the vacuolated cells are chiefly silicone oil laden macrophages. The vacuolated cells in F$_6$H$_8$ related PVR membranes are macropores and also appear to be laden with tamponade agent. In this respect, the histopathology of PVR membranes developing behind F$_6$H$_8$ is similar to retrosilicone oil membranes.

Silicone oil causes cataract. Examination of lens capsule from silicone oil filled eyes has revealed silicone oil laden macrophages adherent to lens capsule. One of the lens capsules in our study exhibited adherent, presumed F$_6$H$_8$ laden, macrophages together with RPE and this specimen was the only one of the three capsular biopsies in which the associated lens epithelial cells showed a fibroblastic phenotype. It is interesting to speculate that macrophage and/or RPE derived factors induced the phenotypic shift. However, this retrolental membrane formed in an eye which had initially contained silicone oil. Moreover, the duration of F$_6$H$_8$ fill was 21 weeks (much longer than in any of the other eyes from which our specimens were obtained). Therefore, it is possible that the lens epithelial changes reflect the extended duration of tamponade.

Although our specimen numbers were too small to confirm any statistical associations, duration of tamponade did not seem to influence the development of multinucleated giant cells within the membranes in the present study. Although such cells are reported in ocular tissues where silicone oil has leaked out of the vitreous cavity, multinucleated giant cells are rarely reported in the silicone oil filled vitreous cavity itself. The apparent trend for a greater foreign body reaction to F$_6$H$_8$ than to silicone oil may reflect the lower viscosity of F$_6$H$_8$. The low viscosity of F$_6$H$_8$ would be expected to expedite emulsification. Emulsification of tamponades such as silicone oil has been implicated in supporting macrophage reactions in the vitreous. Moreover, fluorosilicone oil, which also has a greater emulsification rate than silicone oil, is suspected of promoting PVR. Thus, F$_6$H$_8$ emulsification theoretically might accelerate PVR by inducing macrophage influx and the subsequent accumulation of a wide range of macrophage derived, growth promoting factors.

In conclusion, the histological features of the specimens evaluated in our study are not simply those of PVR. In particular, the macrophagic infiltration suggests that there is a biological response to the novel tamponade agent and this response may be related to a surmised propensity of F$_6$H$_8$ to emulsify. F$_6$H$_8$ has promising physical properties as a long term tamponade and further investigations into the pathobiological effects of this agent are warranted.

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