Experimental model of proliferative vitreoretinopathy (PVR) in the vitrectomised eye: effect of silicone oil

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SUMMARY Silicone oil alone or as an adjunct to vitrectomy is widely used for the treatment of retinal detachment complicated by proliferative vitreoretinopathy (PVR). It appears to reduce the tendency for recurrent traction detachment, but the mechanism of this action is obscure. We have studied the effects of silicone oil in an experimental animal model. Twenty five thousand homologous fibroblasts were injected into the aphakic vitrectomised rabbit eye, and traction detachment of the vascularised retina (medullary rays) regularly resulted. Intraocular silicone oil did not influence the development of this experimental detachment.

The treatment of proliferative vitreoretinopathy (PVR) involves the use of vitrectomy and membrane segmentation techniques or the injection of silicone oil. Different success rates have been reported for vitrectomy, but in general surgical results in established PVR remain poor.¹ Although reattachment of the retina can be regularly achieved, detachment frequently recurs, and extensive membrane proliferation leads to inoperable contraction of the retina.

Several techniques have been suggested to augment vitrectomy and prevent recurrent detachment. Most clinical experience has been obtained with a combination of vitrectomy and silicone injection.² Silicone oil reduces the extent of recurrence, but the explanation of this effect is unclear.

We considered that silicone oil might have a direct influence on the proliferation of membranes on the retinal surface. It is known that the growth of cells in culture is dependent upon their shape and adhesion to the underlying substrate³ and that cells in silicone oil 'round up' and cease to proliferate.⁴ Clinical assessment of our hypothesis was obscured by the heterogeneity of PVR. Therefore we studied this possibility using a model of traction detachment in the vitrectomised eye of an experimental animal. The model closely simulates clinical PVR and recurrent traction detachment after vitrectomy.

Materials and methods

VITRECTOMY AND LENSECTOMY

Pigmented rabbits weighing from 2.5-3.5 kg were sedated with intramuscularly administered KCl (20 mg/kg of body weight), acepromazine (2 mg/kg of body weight), and 1.0 ml of atropine sulphate to reduce bronchial secretions. Supplemental analgesia was provided by a retrobulbar injection of 1% lignocaine hydrochloride. The pupil was dilated with 1% tropicamide, 10% phenylephrine, and 1% atropine. The eye was gently proptosed and exposure maintained by taping the lids. Surgery was carried out under clean but not sterile conditions with the magnification of a Zeiss OPMI 6 operating microscope.

Two small conjunctival peritomies were made at the 11 and 4 o'clock positions. A sclerotomy was made with a 20 gauge needle 1 mm posterior to the limbus at the 4 o'clock meridian. A pars plana cannula attached to a solution of balanced salt was inserted via this sclerotomy and secured with a 5-0 Dexon suture. To maintain pupillary dilatation 0.1 ml of 1:1000 adrenaline chloride was injected into the anterior chamber.

The second sclerotomy was made with a 20 gauge needle 1 mm posterior to the limbus at the 11 o'clock position. The needle was directed into the lens and the nucleus partially disrupted. A Shock Phako fragmenter was used to remove the bulk of the lens. The fragmenter was then withdrawn and replaced with the Ocutome probe. The majority of the lens

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cortex was cut and aspirated. However, some peripheral lens cortex remained. The anterior capsule of the lens was preserved, but its epithelium was removed by gently rubbing the capsule with the tip of the Ocutome probe. The Ocutome probe was then moved into the vitreous cavity and an extensive vitrectomy was performed. No attempt was made to peel the cortical vitreous.

The probe was then withdrawn and the sclerotomy closed with an 8-0 nylon suture. A similar suture was used to close the pars plana infusion site. We injected 15 mg of gentamicin sulphate and 1 mg of dexamethasone phosphate subconjunctivally. An ointment containing polymyxin B, bacitracin, and neomycin, and an atropine ointment were administered.

Provided the anterior capsule was intact post-operative inflammation rapidly subsided, and the pupil could be easily dilated. The eyes were observed intermittently by the indirect ophthalmoscope. If no detachment developed, they were submitted for the second stage of the experiment.

FIBROBLAST TISSUE CULTURE

Homologous fibroblasts were established from skin biopsy specimens cultured in Dulbecco's minimal essential medium supplemented with 20% fetal bovine serum, 50 µg/ml gentamicin sulphate, 10 000 U/ml penicillin and streptomycin, and 5 µg/ml of amphotericin B. The techniques were similar to those described previously.⁵ The cultures were incubated

at 37°C in a humidified atmosphere of 5% carbon dioxide in air. At confluence the cultures were trypsinised, centrifuged, and resuspended in culture media. Aliquots of cells were removed to determine cell concentration in a Coulter counter and to determine cell viability by the trypan blue exclusion test. Viability was never below 95%. The reliability of the dose was estimated by means of additional aliquots from the same syringe. The mean value of the desired dose varied by plus or minus 5%.

FIBROBLAST INJECTION

A 20 gauge cannula attached to an infusion line was inserted through the pars plana. A second sclerotomy was made in the superior pars plana. The Ocutome probe was used to polish the anterior capsule and create an optically clear zone. Any remaining posterior cortical vitreous was also excised. At this point the Ocutome probe was removed, and via the same sclerotomy a gas fluid exchange was performed with a flute needle. The vitreous cavity was filled with a mixture of 50% SF₆ and air. The flute needle was then removed and, again via the same sclerotomy, 25 000 homologous fibroblasts in 0.1 ml of culture medium were slowly injected into the gas bubble through a 20 gauge needle. The cells fell through the gas bubble and formed a fluid level on the medullary rays (Fig. 1a). Fifteen eyes which did not have further procedures, for example, injection of silicone, were used as controls. In the remaining 15 eyes the gas-air

Fig. 1 Diagram of the experimental design. (a) Control group: fibroblasts layered on to the retinal surface under a bubble of 50% SF₆. (b) Test group: fibroblasts layered under a globule of silicone oil.

mixture was exchanged for silicone oil (Fig. 1b). The exchange was performed slowly. Silicone oil was carefully layered on to the top of the cells to ensure that no fluid which might contain cells was lost. The exchange was continued until silicone oil appeared at the sclerotomy. At the completion of the surgical procedure the retina was examined with the indirect ophthalmoscope. If retinal tears were found the eye was excluded from the study. We kept the animals sedated and on their side for 2 hours after the cells were placed on the retina.

Thirty animals were successfully prepared for this study. These were examined at regular intervals by the indirect ophthalmoscope. Drawings were made and selected eyes were photographed. All the animals were killed after 28 days and the eyes enucleated. The clinical findings were confirmed by dissection microscopy. The extent of traction detachment was graded as follows: grade 1—localised detachment of part of a single ray; grade 2—total detachment of 1 or 2 rays; grade 3—total retinal detachment. Selected specimens were examined by light microscopy.

Results

After vitrectomy and lensectomy the postoperative inflammation rapidly subsided. The corneas remained clear and pupils could be dilated. In some eyes it was

Fig. 2 Light micrograph of a paraffin embedded section showing fibroblasts (arrowhead) growing on the rabbit medullary ray in an eye originally filled with silicone. The silicone is lost during processing. (Haematoxylin and eosin, $\times 304$).

Fig. 3 Histograms showing the extent of experimental retinal detachment in the control (a) and silicone injected (b) groups. I=local ray detachment. II=total ray detachment. III=ray and retinal detachment.

possible to see, after a period of some days, detachment of the posterior cortical vitreous. In other eyes this was not visible. In some eyes localised traction detachment of the rays appeared to develop in association with detachment of the posterior vitreous. These eyes and those that developed rhegmatogenous retinal detachment were excluded from this study. After the second procedure the eyes again became inflamed, but provided the anterior capsule was intact the cornea remained clear, and the anterior chamber inflammation subsided.

In both the silicone injected and control eyes we noted the development of membranes after a period of a week to 10 days (Fig. 2). Characteristically these began as either localised pucker adjacent to the disc or contraction of the rays in the region of the disc. In 2 control eyes and 3 eyes filled with silicone progressive traction detachment did not occur (grade 1) (Fig. 3). In 13 (86%) control eyes and 12 (80%) eyes filled with silicone further traction detachment subsequently developed. The rays became narrower and gathered together at the disc. The membranes formed primarily along the medullary ray and optic nerve but were also seen developing on residual cortical vitreous at the periphery of the rays. Residual vitreous in this area was difficult to excise before the injection of fibroblasts.

Clinically the membranes produced in both groups appeared to be similar and could be clearly seen forming behind the oil bubble in those eyes that contained silicone. The time sequence and severity of the traction detachment appeared to be the same in the control eyes and in those that contained a permanent globule of silicone. In 10 (66%) control eyes and 11 (73%) eyes filled with silicone the traction detachment remained limited to the medullary rays (grade 2). In the remaining 3 control eyes and one eye filled with silicone total retinal detachment developed (grade 3).

Discussion

In PVR several different cell types make up the epiretinal membranes. These include retinal pigment epithelial cells⁶ and glial cells.⁷ In addition fibroblasts⁸ and macrophages⁹ have been implicated. These heterogeneous cells become transformed into 'fibrocyte-like' cells¹⁰ which are contractile.¹¹ It is the development of sheets of contractile cells on both surfaces of the retina that is responsible for the development of traction detachment in PVR.¹ Although membranes in recurrent PVR after vitrectomy have been less well studied, identical contractile cells are probably responsible.^{7,12}

Culture fibroblasts injected into the rabbit eye form similar contractile membranes.¹³ To ensure that the injected cells formed membranes directly on the retinal surface residual vitreous cortex was excised and the eye filled with a gas bubble before the injection of cells. Using this technique we were able to localise the epiretinal membrane predominantly to the region of the medullary rays and simulate the recurrent surface retinal membrane seen in clinical redetachment after vitrectomy.

We found no clinical difference between the development of membrane in those eyes in which the vitreous cavity was filled with silicone and the control eyes in which the cavity was filled with a mixture of SF₆ and air. In both groups there was little visible change for several days after the fibroblast injection. The first indication of cell growth was frequently the appearance of localised pucker adjacent to the disc or a grey nodule in the centre of the disc. In most of the eyes of both groups progressive traction detachment of the retina developed over a period of one week to 10 days culminating in gross elevation of both medullary rays, but in the majority of cases without detachment of the surrounding retina.

Was the silicone oil in contact with the developing fibroblasts? The assessment of the size of intraocular bubbles is difficult,¹⁴ but in most cases it was impossible for us to visualise the lower extent of the bubble by the indirect ophthalmoscope. This implies that the vitreous cavity was at least 80–90% filled with silicone oil. Furthermore, the medullary ray region where the traction detachment develops is slightly dorsal in the rabbit eye. Silicone oil is lighter than aqueous fluid and rises to contact this region of the retina with the animal in the upright position. In the eyes filled with silicone oil characteristic shining reflexes from the traction detachment were observed.

This experiment confirms an earlier experiment¹⁵ in which larger numbers of cells were injected directly into a silicone globule. Both imply that silicone oil has no direct influence on the development of traction detachment. These results reinforce our clinical impression² that the therapeutic value of silicone oil is explained by mechanical factors, possibly the permanent closure of retinal breaks.

We thank Kate Borkowski for technical assistance and Susan Gertson for secretarial services.

This study was supported in part by Core Grant EYO3040. The animals used in this study were maintained in animal care facilities fully accredited by the American Association of Laboratory Animal Science.

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