Trypan Blue Staining of Epiretinal Membranes in Proliferative Vitreoretinopathy

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Objective: To determine whether trypan blue staining facilitates epiretinal membrane (ERM) removal in proliferative vitreoretinopathy.

Methods: In 10 patients undergoing vitrectomy for proliferative vitreoretinopathy, ERM peeling was performed without staining the tissue, until no additional ERM were clearly visible. Then, after a fluid-air exchange, 0.06% trypan blue solution was applied onto the retinal surface. After 1 minute, all excess dye was removed and, after an air-fluid exchange, ERM peeling was completed. Excised ERM specimens were analyzed by transmission electron microscopy.

Main Outcome Measures: For each patient, the efficacy of trypan blue staining of ERMs during surgery was scored.

Results: In all patients, intraoperative staining of ERMs with trypan blue was found to be a useful adjunct, since the dye consistently improved direct visualization and delineation of ERMs and facilitated ERM removal. A clear contrast was created between the stained ERM and the nonstaining, underlying retina. Electron microscopy showed that only ERM tissue was removed. No adverse reactions related to the use of the dye were observed up to 3 months after surgery.

Conclusions: Trypan blue may be an important new tool in the surgical management of proliferative vitreoretinopathy, since it may allow a more complete and safer ERM removal.
The completeness of removal of tractional membranes is one of the most important prognostic factors influencing the outcome of PVR surgery.4 However, ERMs are often poorly visible because of their transparency, and a mild sheen or atypical wrinkling of the underlying retina may be the only indirect clue of their presence. When ERMs are visible, their actual extent may be much greater than that expected from their ophthalmoscopic aspect.

Recently, trypan blue staining of the anterior lens capsule was introduced to facilitate the capsulorrhexis during phacoemulsification procedures in the absence of a red fundus reflex.5 To our knowledge, no adverse effects have been reported after the intraocular use of the dye. We therefore speculated that trypan blue could have a use in posterior segment surgery.

Before our clinical study was conducted, the biocompatibility of 0.06% trypan blue was evaluated in vitro by an independent laboratory (BioScan, Laboratory for Medicines and Devices, Belgium) and in vivo in a rabbit eye.6 No adverse effects were observed.

PATIENTS AND METHODS

The study was approved by an institutional review board of the University of Leuven, Leuven, Belgium. Ten patients were enrolled (Table). All patients had advanced PVR (stage C3 and more) after rhegmatogenous retinal detachment or complicated posterior segment surgery, and all agreed to the study by informed consent.

In each patient, ERM peeling was performed, until no residual membranes could be clearly observed under wide-angle viewing conditions (Zeiss operating microscope with EIBOS wide-angle viewing system; Zeiss; Weesp, the Netherlands). A complete fluid-air exchange was then performed, and 0.5 mL of 0.06% trypan blue in a sodium phosphate buffer (VisionBlue; Dutch Ophthalmic Research Center, Zuidland, the Netherlands) was injected in the vitreous cavity close to the retinal surface through a blunt-tipped needle. The eye was rotated in different directions to disperse the dye over the peripheral retinal surface. After 1 minute, all excess dye was carefully removed with a back-flush needle and air was exchanged with fluid.

Removed ERM membranes were immediately fixed in 2.5% glutaraldehyde–0.1M phosphate buffer. After postfixation in 1% osmium tetroxide–0.1M phosphate buffer, the specimens were processed for routine transmission electron microscopy.

All patients were examined at days 1, 2, 3, 10, and 21 after surgery, and then at 2- to 4-week intervals.

### Characteristics and Outcome of Patients*

<table>
<thead>
<tr>
<th>Patient No./Sex/Age, y</th>
<th>Previous Surgical Procedures (Indication for Surgery)</th>
<th>Preoperative VA</th>
<th>Stage of PVR†</th>
<th>Surgical Procedure in Which Trypan Blue Was Used</th>
<th>Follow-up, wk</th>
<th>VA at Last Visit</th>
<th>Status of Retina at Last Visit</th>
</tr>
</thead>
<tbody>
<tr>
<td>1/F/66</td>
<td>1 × Vitrectomy (vitreous hemorrhage in diabetic patient)</td>
<td>LP (poor projection)</td>
<td>D1</td>
<td>Vitrectomy with silicone oil</td>
<td>12</td>
<td>LP</td>
<td>Attached, ischemic retinopathy</td>
</tr>
<tr>
<td>2/M/67</td>
<td>1 × Vitrectomy with air (rhegmatogenous RD) 1 × Vitrectomy with silicone oil (PVR)</td>
<td>10/200 C4</td>
<td>Vitrectomy with silicone oil removal</td>
<td>12</td>
<td>20/200</td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>3/F/62</td>
<td>Diagnostic vitrectomy (endophthalmitis)</td>
<td>1/200 D1</td>
<td>Vitrectomy with silicone oil</td>
<td>6</td>
<td>5/200</td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>4/M/66</td>
<td>Vitrectomy with SF₆ (rhegmatogenous RD)</td>
<td>20/100 C3</td>
<td>Vitrectomy with silicone oil</td>
<td>9</td>
<td>20/60</td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>5/M/63</td>
<td>None 4 × Vitrectomy (rhegmatogenous RD) 4 × Vitrectomy with silicone oil (PVR)</td>
<td>3/200 C4</td>
<td>Vitrectomy with silicone oil</td>
<td>8</td>
<td>20/100</td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>6/F/70</td>
<td>1 × Buckling procedure (rhegmatogenous RD) 1 × Vitrectomy with silicone oil (PVR)</td>
<td>0.5/200 C4</td>
<td>Vitrectomy with silicone oil</td>
<td>7</td>
<td>3/200</td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>7/M/67</td>
<td>1 × Vitrectomy with silicone oil (multiple retinal tears)</td>
<td>3/200 C3</td>
<td>Vitrectomy with silicone oil</td>
<td>9</td>
<td>20/60</td>
<td>Elevated edge of inferior retinotomy</td>
<td></td>
</tr>
<tr>
<td>8/M/55</td>
<td>3 × Vitrectomy with silicone oil (PVR) 1 × Vitrectomy with silicone oil removal 1 × Vitrectomy with silicone oil (PVR, hypotony)</td>
<td>LP (poor projection)</td>
<td>C4</td>
<td>Vitrectomy with silicone oil</td>
<td>7</td>
<td>LP</td>
<td>Attached, choroidal folds (hypotony)</td>
</tr>
<tr>
<td>9/F/73</td>
<td>1 × Vitrectomy with silicone oil (PVR)</td>
<td>2/200 C4</td>
<td>Vitrectomy with silicone oil</td>
<td>10</td>
<td>5/200</td>
<td>Attached</td>
<td></td>
</tr>
<tr>
<td>10/M/70</td>
<td>1 × Vitrectomy with silicone oil (rhegmatogenous RD)</td>
<td>20/200 C3</td>
<td>Vitrectomy with silicone oil</td>
<td>3</td>
<td>20/60</td>
<td>Attached</td>
<td></td>
</tr>
</tbody>
</table>

*VA indicates visual acuity; PVR, proliferative vitreoretinopathy; LP, light perception; RD, retinal detachment; and SF₆, sulfur hexafluoride.
†As classified by the Retina Society Terminology Committee.³
cal Device Evaluation, Bilthoven, the Netherlands): cyto-
toxicity, extract, 24-hour end-point dilution tests were con-
ducted according to the International Standardization
Organization (ISO) 10993 and European Norm (EN) 30993
(H. W. B. Jansen, PhD, unpublished data, 2000). Retinal
tissue changes after long-term exposure to trypan blue were
also evaluated in an in vivo rabbit model. In that study, no
tissue changes were detected with light and electron mi-
croscopy after continuous exposure of 0.06% trypan blue
to the retina for 1 month, whereas high concentrations of
the dye were associated with tissue changes in the inferior
retinal quadrant.6

In the present study, trypan blue was found to cre-
ate a useful contrast between the ERM and the nonstain-
ing retina, thereby clearly delineating the extent of the
ERM. This enabled a more complete removal of the ERMs,
since ERMs that were unsuspected before injection of the
dye were clearly visualized. Because the margins of the
membranes were better delineated, the risk of inadver-
tent damage to the retina was also minimized.

Trypan blue was particularly useful in visualizing
ERMs in long-standing and/or recurrent PVR. In contrast,
in cases of early PVR with a majority of fresh, immature
membranes, the density of trypan blue staining of the ERMs
was found to be highly variable. Trypan blue also proved
to be a helpful tool to assess whether the retinal surface was
completely free of membranes at the time of silicone oil re-
moval. During this procedure, trypan blue was applied to
the retina after aspiration of the oil. The absence of stain-
ing of the retina at this stage supported our decision that
silicone oil removal was safe to perform.

Recently, we also detected that trypan blue and indo-
cyanine green may have complementary staining proper-

Figure 1. Intraoperative view of a poorly delineated epiretinal membrane in proliferative vitreoretinopathy before (A) and after (B) trypan blue staining (digitally processed images).

Figure 2. Margin of this previously unsuspected epiretinal membrane clearly visualized after staining with trypan blue, which facilitated safe and complete dissection from the retinal surface (digitally processed image).

Figure 3. Electron micrograph of an epiretinal membrane stained with trypan blue, showing detail of cellular components (nucleus [asterisk]) and pigment granules (arrows).
ties at the vitreoretinal interface in PVR: although trypan blue shows high affinity for mature ERMs, indocyanine green binds more selectively to the internal limiting membrane but may also stain some epiretinal PVR membranes (data not shown). A double staining technique with trypan blue and indocyanine green proved useful in patients with idiopathic premacular fibrosis (P.S., unpublished data, 2001).

In conclusion, trypan blue staining of ERMs was found to be a useful adjunct in the surgical management of PVR, since it allows a more complete and safer removal of ERMs. Long-term clinical studies are needed to determine whether this novel technique will ultimately result in a better anatomic and functional outcome of PVR surgery.

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REFERENCES