Double vital staining using trypan blue and infracyanine green in macular pucker surgery

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Aims: To study the clinical properties of double vital staining in premacular fibrosis, facilitating complete removal of all epiretinal tissue.

Methods: In a two step surgery, the epiretinal pucker was removed after staining with trypan blue, whereas after the inner limiting membrane was peeled after staining with infracyanine green.

Results: In all 30 patients, a separate epiretinal layer and inner limiting membrane were removed from the macular area. Pathological examination showed different histological properties of the removed layers. An increased visual acuity was measured in 26 patients, and a slightly decreased visual acuity in one patient.

Conclusion: The described double staining technique could be a novel valuable tool that may help to achieve optimal anatomical and functional recovery after surgery for premacular fibrosis.
procedure was recorded using a digital video imaging system from CIT Engineering (Geel, Belgium, http://www.citeng.com).

The intraocular use of trypan blue was approved by the ethics committee of the UZLeuven.

METHODS

In this study, 30 patients were elected with decreased visual acuity secondary to the formation of premacular fibrosis. Preoperative examination included visual acuity measurement, biomicroscopical evaluation, dilated fundus examination, optical coherence tomography of the macular area, and fluorescein angiography in elected patients if other abnormalities were suspected. Informed consent was obtained from all patients. When lens opacities were present and the patient was over 65 years of age, the vitrectomy was preceded by phacoemulsification of the lens and intraocular lens implantation. A three port vitrectomy with vitreous base shaving was performed first, and a fluid/air exchange performed, with removal of most of the intraocular fluid using a blunt back flush needle. A 0.15% solution of trypan blue was then aspirated in a 2 ml syringe, and connected to a blunt tipped needle. With the needle opening close to the retinal surface, approximately 200–500 µl of the solution was sprayed over the macular area in the air filled eye. The air in the vitreous cavity prevented the trypan blue dye from diffusing away from the posterior pole. After 2 minutes, most of the dye was removed with a back flush instrument using the positive air pressure (30 mm Hg), whereafter an air/fluid exchange was performed, to aspirate the remaining dye. The macular epiretinal membrane could then be observed as a light blue stained entity, sharply demarcated from the non-stained neural retina (Fig 1). The stained fibrotic tissue was grasped with an intraocular forceps, and carefully removed from the posterior pole (Fig 2). In the next phase of the surgical procedure, approximately 500 µl of the infracyanine green solution was injected over the posterior pole, while the infusion line was closed. After 2 minutes, the infusion line was reopened, and the excess of the dye removed. The ILM was then incised using a sclerotomy knife, grasped and removed from the posterior pole in a rhexis-like manner (Fig 3).

RESULTS

In this study, 30 patients were treated for the formation of an idiopathic epiretinal membrane using the above described double staining technique. The average age was 70 years (range 52–80), with equal M/F distribution (16/14). The average follow up time was 23 weeks (range 4–40). Preoperative visual acuities ranged from 20/800 to 20/30 (Fig 4). In this patient group, 25 pukers were idiopathic, four developed after previous vitrectomy for retinal detachment, and one patient underwent a previous buckling procedure for retinal detachment. The vitrectomy was combined with a phacoemulsification procedure and IOL implantation in 17 patients (57%), nine patients (30%) were already pseudophakic before the vitrectomy. In two patients, postoperative lens opacification occurred, requiring cataract surgery. In 14 patients (47%), a gas tamponade with SF6 (15%) was used.

A complete removal of the visualised epiretinal tissue and underlying ILM was obtained in all patients. Sometimes, small capillary haemorrhages occurred during the removal of the ILM. These haemorrhages disappeared within 24 hours after surgery.

To make certain that the two stained layers corresponded respectively to the epiretinal and internal limiting membrane, both peeled tissue samples were collected in different fixation tubes and examined using electron microscopy (Fig 5). Figure 5A shows a detail of the epiretinal membrane that had been removed after staining with trypan blue. The pucker contained multiple fibrous astrocytes (intermediate type intracellular filaments, interdigitating cytoplasmic processes, and junctional complexes) with myofibroblast differentiation and collagen fibres. The underlying ILM that had been removed after staining with ICG is shown in Figure 5B. The peeled ILM contained scrolled and layered basal membrane material with very few fibrocytes.

All patients were examined at day 1, 2, 3, and 10 or 14 after surgery. Most patients (60%) reported decreased metamorphopsia in the early postoperative period. In 28 patients (93%), visual acuities returned to preoperative values within 14 days after surgery. In one patient, corneal oedema was still

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DISCUSSION

We have shown that trypan blue and infracyanine green can be used in the same procedure because of the complementarity of the staining properties of both dyes: trypan blue shows affinity for epiretinal membranes as seen in idiopathic premacular fibrosis and less affinity for ILM, as seen in ILM staining during surgery for macular holes (personal observation). In contrast, green dyes are not very useful for epiretinal membranes staining while excellent for ILM visualisation.

It is generally accepted that complete epiretinal tissue removal from the posterior pole is a prerequisite for maximal functional recovery after surgery. Since the epiretinal membrane tissue often extends further than expected by ophthalmoscopY, the described technique using trypan blue staining could be a useful tool to facilitate its complete removal. After epiretinal membrane removal, in most cases the retinal surface still showed visible striae with an overlying folded ILM. Residual tangential traction on the neural retina by folds in the ILM may contribute to the persistence of metamorphopsia and macular oedema that is sometimes observed after macular puck surgery. Rips in the ILM were also seen after epiretinal membrane removal, probably secondary to prolonged traction exerted by the overlying macular puck tissue or by adhesion to the removed overlying epiretinal tissue. ICG staining of the ILM was also very useful to facilitate its complete and safe removal.

The safety of the trypan blue staining has been evaluated in a rabbit experimental model injecting trypan blue in a gas filled eye. Despite long term intraocular application, no adverse effects were detected 1 month after injection of the low concentration solution of trypan blue (0.06%). Toxic effects to the retina were only seen after 1 month of exposure to higher concentrations. Toxic effects of trypan blue on the retinal pigment epithelial cells were not observed in vitro after a 5 minute contact time even at a concentration of 0.3%.

The safety and usability of intraocular indocyanine green use was previously tested in rabbit experiments, in cadaveric eyes, and in surgery for macular holes. Recent reports have demonstrated a possible toxic effect of indocyanine green on retinal pigment epithelial cells in vitro and after surgery for macular holes. This toxic effect is probably due to the hypo-osmolarity of the used solvent, and is less likely to occur when infracyanine green dissolved in glucose is used for staining the ILM. Therefore, the latter dye was used in this study. Moreover, since during surgery for epiretinal membrane no direct contact between the used dyes and the retinal pigment epithelium is expected, toxic effects are less likely to occur.

During follow up we did not observe any ophthalmoscopic signs of adverse effects of the staining method as used in our patients.

In conclusion, we believe that the described double staining technique could be a novel valuable tool during surgery for premacular fibrosis.

ACKNOWLEDGEMENTS

Proprietary and financial interest: Dr Gerrit R J Melles has proprietary and financial interest in commercially available solutions of trypan blue for intraocular use (VisionBlue and MembraneBlue, DORC, Zoeland, Netherlands).

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