SCIENTIFIC REPORT

Vitreoretinal surgery using bromphenol blue as a vital stain: evaluation of staining characteristics in humans

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Objective: To evaluate the staining characteristics of bromphenol blue used during vitreoretinal surgery in humans.
Patients and methods: 13 patients with epiretinal membranes were included. Before and after surgery a complete clinical examination including best corrected visual acuity, funduscopy, fluorescein angiography, OCT (Stratus), Goldmann perimetry and multifocal ERG as well as photography of the macular area was performed. Bromphenol blue was used in concentrations of 0.2% in most patients. Removed epiretinal tissue was evaluated using electron microscopy.
Results: Using dye concentrations of 0.2% a good demarcation of epiretinal membranes was seen in 11/13 patients. Staining of vitreous remnants at the vitreous base was seen in all patients. No dye-related adverse events were seen during follow-up in the functional tests (VA, ERG, perimetry) performed. Histological evaluation of epiretinal membranes showed unremarkable aspects of epiretinal cellular layers and unremarkable retinal surface of the internal limiting membrane (ILM).
Conclusion: Bromphenol blue appears to be a very helpful and safe tool in posterior segment surgery. The staining characteristics need to be further evaluated in prospective study settings and larger numbers of patients.

Vital dyes potentially facilitate vitreoretinal surgery by visualising nearly transparent structures such as the internal limiting membrane (ILM) and epiretinal membranes (ERM). Especially for surgeons at the beginning of their learning curve the use of dyes may help to reduce the risk of mechanical trauma and damage of underlying structures, such as the nerve fibre layer, and allow for a more complete removal of the target structure. Two dyes are currently available for intraocular application: indocyanine green (ICG)[1] and trypan blue.[2] Whereas ICG has been shown to selectively stain the ILM,[3] trypan blue is mainly used to visualise ERM.

ICG became the subject of ongoing discussion[4–8] as clinical and experimental data suggested dye-related toxicity, leading to less favourable functional outcome after macular surgery. As the underlying mechanisms of action as well as the safety margins of ICG are not completely understood so far the applicability of ICG seems to be limited. Although no significant clinical adverse events have been reported for trypan blue in humans, chronic and acute toxic effects have been seen in animals and cell culture models.[9 10]

Therefore there appears to be a need for alternative dyes, providing both satisfying staining characteristics and a good safety profile. We initiated an investigation on novel dyes to assess both potential toxic effects and staining characteristics in different cell culture and animal models.[11–13] As a result of these studies, bromphenol blue appeared to be a promising candidate for the application in humans.

In the present report, we describe the first experiences with this novel dye obtained during vitreoretinal surgery for tractive maculopathies such as macular holes and macular pucker.

METHODS

The study was approved by the local ethics committee and institutional review board, and written informed consent was obtained from all patients. Thirteen patients with macular pucker, seven males and six females with a mean age of 65, were included in the present study.

Preoperatively and postoperatively, patients underwent a complete clinical examination including measurement of best corrected visual acuity (VA), slit-lamp examination, tonometry,

Table 1 Complete data of the histological findings in the membranes peeled off during surgery

Features of tissue fragments	No. of cases (n = 8)
Inner limiting membrane	
Present	7
Not present	1
Native vitreous collagen	
None	4
Some	1
Continuous layer	3
Newly formed collagen	
None	2
Some	2
Irregular masses	4
Fibrous long spacing collagen	
Present	2
Not present	6
Cellular distribution	
None	1
Single cells	0
Monolayer	1
Multilayer	6
Presence of	
Fibrous astrocytes	3
Fibroblasts	7
Myofibroblasts	7
RPE cells	1
Macrophages	0
Predominating cell type	
None	1
Fibrous astrocytes	0
Fibroblasts	4
Myofibroblasts	3
RPE cells	0
Macrophages	0
Retinal fragments at the ILM	
None	1
Small fragments	4
Larger fragments	3

Abbreviations: ERM, epiretinal membranes; ICG, indocyanine green; ILM, internal limiting membrane; VA, visual acuity

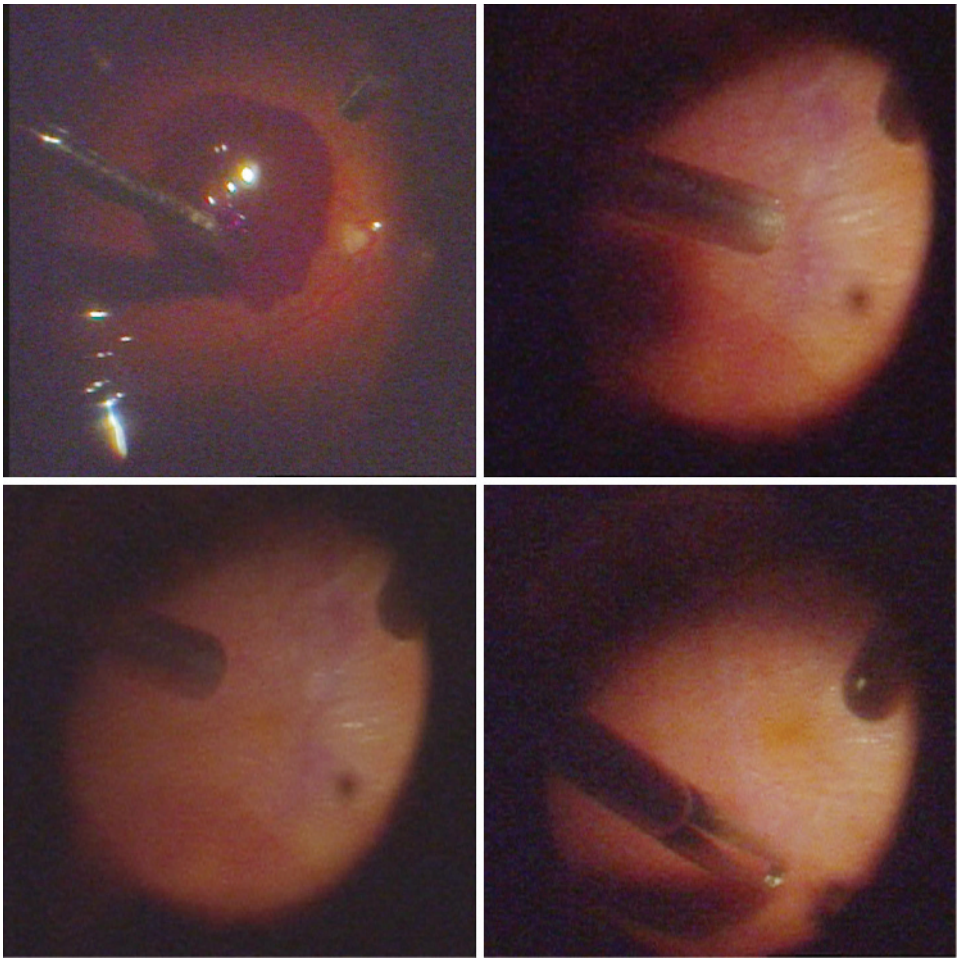

Figure 1 Peeling of an epiretinal membrane after staining with bromphenol blue.

funduscopy using a 78 diopter lens (Volk Optical, Mentor, OH, USA), fluorescein angiography, OCT (Stratus), Goldmann perimetry, multifocal ERG and fundus photograph. Patients were seen one day before surgery and then in six-week intervals. Postoperatively, ERG and Goldmann perimetry were performed at the six-week follow-up visit and were not repeated if unremarkable. All examinations were performed by one of the authors (RS).

Bromphenol blue was dissolved and diluted using BSS plus and sterilised using a 0.22 µm syringe filter and dye concentrations of 0.02% and 0.2% were then injected into the eye.

Vitrectomy consisted of a standard three-port pars plana vitrectomy as described in previous reports.[4 5] Before injection of the dye, a fluid air-exchange was performed to avoid an uncontrolled dye distribution. Then, a few drops of the dye were applied over the macular area. After a period of one minute, the dye was completely washed out by irrigation. The staining characteristics were then evaluated by the surgeon and an additional examiner (CH). This was followed by removal of epiretinal tissue and the ILM using an end-gripping forceps. After the removal of epiretinal tissue, no second dye injection was performed in this series of patients. Surgical procedures were performed by one of the authors (AK).

All epiretinal tissue removed during surgery was harvested and immediately prepared for ultrastructural analysis as reported previously.[4] Ultrathin sections were obtained from all specimens, contrasted with uranyl acetate and lead citrate, and analysed using a Zeiss EM 9 electron microscope (Zeiss Jena, Germany). Histological evaluation of specimens was performed by one of the coauthors who did not observe the surgical procedure and had no access to other patient data (RGS).

RESULTS

We included 12 patients with idiopathic macular pucker and one patient with ERM formation in association with proliferative diabetic retinopathy. As no staining of epiretinal tissue was seen during surgery using a dye concentration of 0.02% in the first patient, all remaining patients were operated on using concentrations of 0.2%. In patients with clinically visible ERM satisfying staining of the membrane was seen in 11 of 13 cases (fig 1). In two cases the staining effect was rather weak. We had the impression that the staining effect varied depending on the thickness of the ERM. Less staining was noted in cases where it appeared difficult to remove an ERM, suggesting that the pathological alterations were more pronounced within the retina and not on the retinal surface. We did not observe sufficient staining of the ILM in our patients with ERM. Staining of remnant vitreous in the periphery at the vitreous base could be seen in all cases.

Overall patients' VA increased from 0.16 preoperatively to 0.32 postoperatively. No loss of VA was seen. No dye-related complications were observed in any functional test performed. We found no affection of the amplitudes in postoperative ERG examinations nor did we observe peripheral visual field defects attributable to the dye. In one patient a visual field defect occurred as a result of intraoperative damage to the retina with the end-gripping forceps following a head movement of the patient during peeling and one case of peripheral retinal detachment was seen which was treated successfully by a second vitrectomy with gas tamponade. In the patient with proliferative diabetic retinopathy the visual field was affected following panretinal photocoagulation before surgery. On follow-up of 3 months, a decrease of mean retinal thickness measured by OCT from 465.8 µm to 361.75 µm was seen.

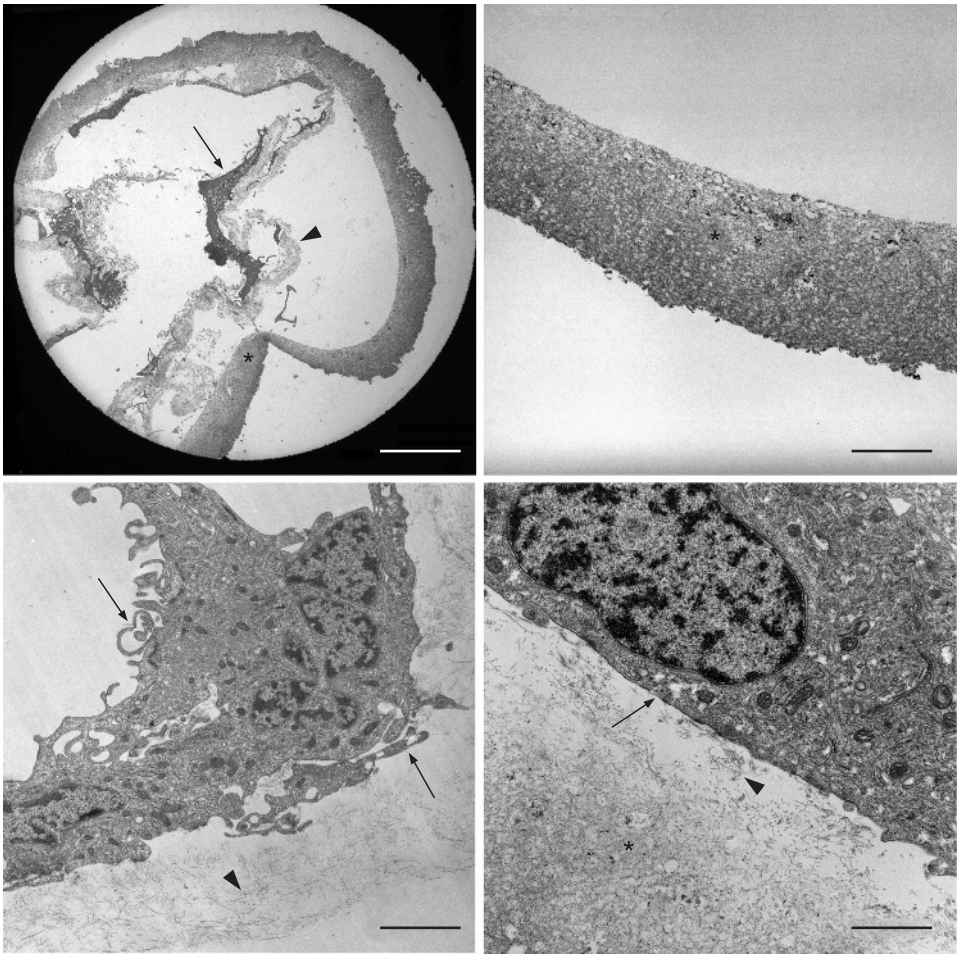

Figure 2 Transmission electron micrographs of the internal limiting membrane (ILM) and epiretinal tissue removed by bromphenol blue-assisted peeling. The ILM was present in most specimens, with native vitreous collagen being interspersed between the ILM and epiretinal cells in many specimens. Newly formed collagen was also seen irregularly distributed between the ILM and cellular elements as well as embedded in areas with cellular proliferation. We observed fibrous astrocytes, fibroblasts, myofibroblasts and RPE cells. *Top left*, en-bloc peeling of an irregular layer of cells (arrow) and collagen (arrowhead) directly attached to the vitreal side of the ILM (asterisk) (original magnification, x1000; bar = 10.1 μm). *Top right*, the ILM devoid of cells and collagen (original magnification x3600, bar = 2.8 μm). *Bottom left*, densely packed cells with numerous microvillous processes (arrows) on a layer of native vitreous collagen in areas without contact to the ILM (original magnification, x3600; bar = 2.8 μm). *Bottom right*, fibroblast (arrow) with cellular nucleus and abundant endoplasmatic reticulum at the ILM (asterisk), newly formed collagen (arrowhead) sparsely distributed between fibroblast and ILM (original magnification, x9500; bar = 1.0 μm).

Epiretinal membranes of 8 patients following vitrectomy were evaluated using microscopy. The histological evaluation focused on the appearance of the epiretinal cellular layers and the amount of cellular debris on the retinal surface of the ILM (fig 2). Epiretinal cells all had maintained their cellular integrity. Small cellular fragments at the retinal surface of the ILM were frequently noted (table 1).

DISCUSSION

With respect to reports on adverse effects following the use of ICG,[4–6] which is mainly used to stain the ILM, but also for trypan blue,[9 10] it seems reasonable to look for potential new dyes with a better safety profile and reliable safety margins for intraocular application. Several dyes are currently under investigation experimentally[11–15] and in humans.[16]

One dye, bromphenol blue, revealed promising staining characteristics and an excellent safety profile[11 12] and was therefore further evaluated in humans in the present preliminary case series. We observed that the staining effects of bromphenol blue varied with less staining noted in cases where hardly any tissue could be peeled off. Otherwise, a nice demarcation of the membrane was observed. As a consequence, we hypothesise that bromphenol blue might serve as a diagnostic tool intraoperatively, helping to avoid excessive peeling in cases of predominant intraretinal abnormalities.

We also observed a strong staining of vitreous remnants at the vitreous base in all cases. This seems to be relevant as a complete removal of the vitreous is crucial for surgical success in many conditions. Vitreous staining might be a very helpful tool, especially for less experienced surgeons, during surgery for retinal detachment, where a thorough removal of vitreous in the area of the retinal break is important, or tractive retinopathy in proliferative diabetic retinopathy. In addition, the detection and complete dye-assisted removal of vitreous remnants on the retinal surface may provide enough relief of tractional forces, for example in smaller (stage II) macular holes, and ILM peeling might therefore not always be necessary.

Histological evaluations of ERM removed during surgery showed morphological features which were in line with previous reports in the literature.[17] Of note, we did not observe a disruption of epiretinal cells or great amounts of cellular debris on the retinal surface of the ILM as a sign of dye-related toxicity as reported previously.[5]

A limitation of the present investigation is the rather small number of patients and the lack of control groups including other dyes or conventional surgery without staining. The staining properties should therefore be further evaluated in prospective studies incorporating larger numbers of patients. In addition, higher dye concentrations might help to enhance the staining properties. Nevertheless, one might argue that another dye (trypan blue) is already available for ERM staining. However, although adverse effects of trypan blue have not been reported following clinical use in humans, several reports have indicated chronic toxic effects of trypan blue in experimental settings in vivo and ex vivo.[9 10 18–20] Therefore, investigations into alternative dyes seem justified and necessary.

In summary, bromphenol blue provided good staining properties in epiretinal membranes and vitreous and showed a very good safety profile intraoperatively and during follow-up. Statements on long-term safety can not be made before a longer period of review. In addition, further studies are required

to compare the functional outcome of bromphenol blue-assisted vitrectomy to other dyes such as trypan blue or conventional surgery without staining.

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Dr Haritoglou applied for a patent for the dye and its use.

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