Review Article

Chromovitrectomy: Update

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Abstract

The basic concept for the application of vital dyes during vitreoretinal surgery is to assist in highlighting preretinal membranes and tissues which are very thin and semitransparent and thus difficult to detect. The vital dyes may be classified according to different criteria, where the most commonly applied includes chemical classification. In ophthalmic surgery, vital dyes are widely used in cataract and vitreoretinal surgery. The vital dyes, indocyanine green, infracyanine green, and brilliant blue stain the internal limiting membrane, and trypan blue and triamcinolone acetonide help to visualize epiretinal membranes and vitreous, respectively. This review exhibits the current literature regarding the properties of vital dyes, techniques of application, indications, and toxicities during vitreoretinal surgery and, also suggests that the field of chromovitrectomy represents an expanding area of research.

Keywords: Chromovitrectomy, Vital dyes, Indocyanine green, Internal limiting membrane, Vitreoretinal surgery

Introduction

The field of vitreoretinal surgery has grown over the last several decades. Scientific advances improved our understanding of disease pathology and new surgical adjuncts and techniques have decreased surgical time and increased success rate. One of the innovations in vitreoretinal surgery over the past 10 years has been the introduction of vital dyes to improve the visualization of preretinal tissues and membranes.1–4

The term “chromovitrectomy” refers to the use of vital dyes during vitreoretinal surgery to assist in the identification of preretinal tissues and membranes.5 In 2000, the modern approach was first introduced, when the dye indocyanine green (ICG) was used to stain the thin semitransparent internal limiting membrane (ILM), and it is currently the most commonly used surgical dye in ophthalmology. However, over the past few years, controversial evidence has accumulated indicating that these dyes may have harmful effects. Following initial experience with ICG, clinical and experimental studies demonstrated signs of the retinal toxicity of ICG, which stimulated research on alternative dyes for chromovitrectomy. Some additional alternative biostains, including trypan blue (TB), or brilliant blue (BriB), and patent blue (PB), have been added to the surgical collection for chromovitrectomy.6 This review presents the latest data on chromovitrectomy in regard to the biochemical properties, indications, and clinical experience with various vital dyes available for chromovitrectomy.

Biochemical pharmacology

Vital staining refers to the coloration of living cells or tissues. Dyes are organic molecules containing chromophores. A chromophore is the part of a molecule responsible for its color.7

The staining agents may be classified according to the chemical classification. Some of the groups of dyes already used in chromovitrectomy are: (1) azo dyes; (2) arylmethane dyes; (3) cyanine dyes; (4) xanthene dyes; and (5) colored corticosteroids.
Azo dyes are a class of synthetic organic dyes with nitrogen in the azo form of –N=–N– in their structure. TB is an anionic hydrophilic azo dye which has the molecular formula C_{32}H_{42}N_{6}Na_{3}O_{14}S_{4} and a molecular weight of 960 Da. TB is so-called because it can kill trypanosomes, the parasites that cause sleeping sickness.\textsuperscript{6} TB crosses the cell membranes of dead cells only, because of that stains dead tissues/cells blue. In ophthalmology, TB has in a preferential manner affinity for the epiretinal membrane (ERM).\textsuperscript{10–13} TB may be commercially available at a concentration of 0.15% for vitreoretinal surgery, called Membrane Blue (DORC International, Zuidland, Netherlands).

Cyanine dyes are a class of dyes containing a –CH– group linking two heterocyclic rings containing nitrogen. The cyanine agent has amphiphilic properties and thereby binds to both cellular and acellular elements in living tissues. ICG is a tricarbocyanine anionic vital dye with a molecular formula of C_{32}H_{42}N_{6}Na_{3}O_{14}S_{4} and a molecular weight of 775 Da. The sterile hydrophilic powder represents a very useful contrast agent in angiography, allowing imaging of choroidal and retinal tissues.\textsuperscript{14–20} For ophthalmology, ICG is commercially available under the names of ICG (Pulsion Medical Systems, Munich, Germany; 25- and 50-mg vials), ICG Indocyanina Verde (Ophthalmos, São Paulo, Brazil; 5-, 25-, and 50-mg vials), Diagnogreen (Daiichi Pharmaceutical, Tokyo, Japan; 25-mg vial), and IC-Green (Akorn, Buffalo Grove, USA; 25-mg vial).

Infracyanine Green (IfCG) is a green dye with a chemical formula and pharmacologic properties similar to those of ICG. However, IfCG has a clear advantage over ICG because it is synthesized without sodium iodine, as it is believed that iodine damages the cornea and retina.\textsuperscript{21–25} On the other hand, iodide-free IfCG is not water soluble and has to be dissolved in 5% glucose solvent IfCG.\textsuperscript{26–28} It is commercially available under the brand name of Infracyanine (Laboratoires SERB, Paris, France; 25-mg vial).

Arylmethane dyes are stains which are formed by one carbon linked to benzene or naphthalene groups; they are commonly used in modern inks. BroB is a blue anionic arylmethane compound which has the chemical formula of C_{27}H_{20}N_{2}NaO_{14}S_{2}. Na and a molecular weight of 854 Da. This dye was reported to stain the ILM and to have no significant in vivo toxicity.\textsuperscript{23–26} The dye gained acceptance for intraocular use under the brand name of Brilliant Peel (Geuder, Heidelberg, Germany), and it is provided in vials containing 2 mg/ml of the vital dye. Another example is Bromophenol blue (BroB) which is an acid–base indicator that has recently been proposed as a promising alternative biostain for vitrectomy, because it has induced no damage in either in vitro or in vivo studies.\textsuperscript{25,26} It has a molecular weight of 670 Da and the chemical formula is C_{19}H_{13}BrO_{3}S. PB is a hydrophilic anionic triarylmethane dye with the chemical formula C_{32}H_{31}N_{2}NaO_{14}S_{2} and a molecular weight of 582 Da. PB has been applied as an off-label agent in vitreoretinal surgery.

Xanthene is a yellow organic heterocyclic compound. Its chemical formula is C_{13}H_{10}O. Xanthene dyes tend to be fluorescent, yellow to pink to bluish red, brilliant dyes. Fluorescein is a xanthene fluorophore with the chemical structure C_{20}H_{22}O_{5} and a molecular weight of 332 Da.

Corticosteroids are hormones produced naturally in the cortex of the adrenal gland, whose derivatives may be synthetically produced to be used as drugs in the treatment of human diseases. Triamcinolone acetonide (TA) is a synthetic insoluble corticosteroid with the empirical formula C_{20}H_{31}FO_{6} and a molecular weight of 434 Da. In ocular surgery, TA has manifested a good staining of the vitreous because of the crystal composition.

### Indications and toxicology

#### Indocyanine green

ICG facilitates ILM removal intraoperatively because it has an affinity for extracellular matrix components of the ILM, such as collagen and fibronectin.\textsuperscript{1,2} ICG had been used in chromovitrectomy for macular hole treatment (Fig. 1).\textsuperscript{45,46} However, Morales et al.\textsuperscript{47} indicated that the effects of ICG are not strongly dose dependent, at least at the physiological concentrations examined. ICG has been found to be associated with the risk of damage to the photoreceptors and RPE cells,\textsuperscript{48,49} atrophy of the retinal pigment epithelium (RPE),\textsuperscript{50} loss of epiretinal cellular integrity,\textsuperscript{51} and cellular toxicity,\textsuperscript{52–55} among other harmful effects. In conclusion, although much evidence suggests that ICG may exert toxic effects on the retina, the staining agent in low doses should be safe for chromovitrectomy.

#### Infracyanine green

IfCG also binds with high affinity to the acellular ILM and facilitates its visualization and peeling similar to ICG. It is marketed as ICG-assisted ILM peeling (Fig. 1).\textsuperscript{56} However, ICG-assisted ILM peeling in DME induced no sign of retinal toxicity by visual acuity measurements when comparing vitrectomy with and without ILM peeling.\textsuperscript{1,2} Although the best indication for ICG use in chromovitrectomy is for ILM staining in MH surgery, ICG has been proposed for better visualization of epi-retinal membranes (ERM) in vitrectomy for proliferative diabetic vitreoretinopathy, idiopathic ERMs, and proliferative vitreoretinopathy (PVR).\textsuperscript{57–59} However, the green dye may stain the acellular ILM better; as for the task of ERM staining, other vital stains may be better.

Regarding the toxicity of ICG, some investigators have reported that the toxic effects of ICG are time and dose dependent.\textsuperscript{60–62} However, Morales et al.\textsuperscript{47} indicated that the effects of ICG are not strongly dose dependent, at least at the physiological concentrations examined. ICG has been found to be associated with the risk of damage to the photoreceptors and RPE cells,\textsuperscript{63,64} atrophy of the retinal pigment epithelium (RPE),\textsuperscript{65} loss of epiretinal cellular integrity,\textsuperscript{66} and cellular toxicity,\textsuperscript{52–55} among other harmful effects. In conclusion, although much evidence suggests that ICG may exert toxic effects on the retina, the staining agent in low doses should be safe for chromovitrectomy.
safer than ICG due to the absence of iodine in its formulation, and has reduced adverse effects.\textsuperscript{29–34,56} IfCG at a concentration of 0.5 mg/ml results in adequate ILM identification and less toxic effects.\textsuperscript{57} No evidence of significant acute toxicity is associated with IfCG.\textsuperscript{47} However, IfCG can be phagocytosed by RPE cells, remaining in the interior of these cells for long periods, with a risk of inducing chronic toxicity.\textsuperscript{34}

Trypan blue

TB usage recommends blue dye application mainly for ERM staining.\textsuperscript{9–17} TB exhibits outstanding affinity for ERM because of the strong presence of dead glial cells within those membranes. I personally use it for immature PVR which may minimize mechanical trauma to the retina during ERM removal and allow the recognition of the whole extent of the ERM. TB in various doses may enhance the ability to detect both the prolapsed vitreous to the anterior chamber and the posterior vitreous remaining in the vitreous cavity, but it is inferior to TA.\textsuperscript{58} Regarding the chronic toxicity of TB, it has been reported that it induces arrest of the cell cycle at G0–G1 via increased expression of p21.\textsuperscript{59} Some researchers showed that a subretinal injection of 0.05% ICG results in a more substantial retinal damage than that associated with subretinal injection of 0.15% TB.\textsuperscript{57}

Brilliant blue

BriB has been introduced as a surgical adjuvant for chromovitrectomy in 2006 and, this dye was reported to stain the ILM and to have no significant in vivo toxicity.\textsuperscript{23,24} Cervera et al.\textsuperscript{60} showed similar outcomes with good ILM staining and clinical results and no signs of toxicity in multifocal ERG. BBG is more hydrosoluble than ICG and IfCG; it would thus penetrate less into the cells and be more easily washed away, leaving less residues after surgery.\textsuperscript{45} For this reason, BriB represents a good alternative for ICG and IfCG in chromovitrectomy due to its suitable affinity for ILM (Fig. 2).

Patent blue

The utility of the PB dye for applications in ophthalmology has only recently been discovered, and relatively little is known about its effects. For this reason, the present findings are of particular interest.\textsuperscript{47} It has a moderate affinity for ERM and vitreous, but a poor affinity for the ILM.\textsuperscript{17} PB affects human retinal function when applied for at least 1 min. However, no irreversible effects on the human ERG were seen even after 2 min of retinal exposure to patent blue. Thus, toxic effects on retinal function after intraoperative short-term application of patent blue 0.48% appear unlikely to occur.\textsuperscript{13} Subretinal injection of TB induced a more significant clinical and histologic damage of neurosensory retina/RPE than did PB or Balanced Salt Solution (BSS).\textsuperscript{19}

Bromophenol blue

BroB has been applied in ocular surgery: the dark-blue stain may represent a novel useful adjunct for both cataract and vitreoretinal surgery. BroB stained the retinal surface and lens capsule at a low concentration (0.2%) with no signs of toxicity, this dye seems to be the most promising candidate for application in humans.\textsuperscript{15} Schuettau et al.\textsuperscript{16} demonstrated that BroB dye tested in his study did not lead to detectable toxic effects in the rat eye, even after prolonged presence within the eye and an observation period of 7 days. Other novel dyes as well as ICG showed toxic effects, such as histologic alteration of the retina and loss of RGCs.

Sodium fluorescein

SF is highly safe for fundus angiography at concentrations of 5–25%. The intravitreal 0.20% SF dye improves the visualization of clear vitreous fibers through a green staining during chromovitrectomy. The clear vitreous can be stained markedly green by SF administered 12–16 h before surgery.\textsuperscript{61} A preoperative diagnostic fluorescein angiography in eyes with active uveitis or diabetic retinopathy may lead to a moderate accumulation of the dye in the vitreous cavity and greenish staining of the vitreous cortex at the vitreoretinal interface.\textsuperscript{62,63} SF 0.6% can safely be used in the vitreous cavity for easy identification of clear uncut vitreous gel for clear vitreous vitrectomy. This enhances efficient excision of this tissue.\textsuperscript{64}

Triamcinolone acetonide

TA produces the best vitreous visibility in comparison to other stains.\textsuperscript{65} The crystals of the steroid adhere to the acellular tissue, thereby enabling a clear contrast between the empty vitreous cavity compared to areas with the vitreous fibers remaining.\textsuperscript{66} The surgical technique for TA application consists of a direct injection of the agent into the vitreous cavity toward the area of interest. TA-assisted removal of internal limiting membrane was used in many cases since the white specks and crystals may deposit over the ILM, thereby facilitating ILM removal (Fig. 3).\textsuperscript{57,66} Crystals of TA have been detected up to 40 days postsurgery with chromovitrectomy for MH surgery. For this reason, some authors suggest that postoperative residual TA could diminish the healing process necessary for MH closure.\textsuperscript{67} Injecting this steroid during vitrectomy for the management of retinal detachment may prevent fibrin reaction and PVR postoperatively.\textsuperscript{70–72} The commonly used formulation of TA, kenalog, is not formulated for the eye, for this reason, there is a risk of pseudooendothalmitis and retina toxicity when injected intravitreally.\textsuperscript{73,74} There have been reports of toxicity of TA
on retinal pigment epithelial cells (RPE) in vitro whereas ex vivo and in vivo studies have not shown any significant toxicity on the retina.

Vital dyes and light sources

Interaction of light from endoillumination source and vital dye may increase or decrease the risk for toxicity. Light-induced retinal toxicity by the endoilluminator is dependent on factors such as the duration of use, type, power, and wavelength of light source. Vital dyes are small chemical substances that pass freely through retinal tissue and may play a role in or exacerbate retinal phototoxicity from intra-operative light exposure. Photosensitizing dyes could enhance phototoxicity by increasing levels of free radicals, creating a photoproduct that could be harmful to retinal cells and shifting light absorbance from one site of the retina to another. In this regard, the dye on the retinal surface could increase the risk for phototoxicity to the neuroretina for light greater than 450 nm, which would not occur without dyes. In addition, dyes in the subretinal space may exacerbate damage to the RPE after exposure to various wavelengths of light. From a surgery perspective, among all light sources analyzed by Costa et al., the greatest overlap was found with integrated laser pathway (Photon Xenon; Synergetics) and halogen (Grieshaber GLS; GLS Corp.), and the least overlap was found with mercury vapor lamp (Photon 2; Synergetics). The lowest overlap values among the dyes were observed with ICG prepared in PSS, followed by IC, which showed low values for all three solvents compared with other dyes.

Dye injection techniques

There are different ways to protect the RPE cells during dye injection in the MH surgery: slow injection of the dye, placing substances over the MH such as sodium hyaluronate, perflourocarbons liquids (PFCL), autologous whole blood, or use of VINCE (vitreoretinal internal limiting membrane color enhancer) which enables selective painting of ILM that needs to be removed without staining perifoveal and peripheral retinae. The most common two ways in dye injection technique are:

1. The “dry method” or “air-filled technique”. This technique consists of removing the fluid in the vitreous cavity by a fluid-gas exchange before dye injection. While the technique has the advantage of concentrating the dye in the posterior pole and avoiding contact at the posterior capsule of the lens, it may expose the retinal surface to a higher concentration of dye to the vitreoretinal interface.

2. The “wet method” or “fluid-filled technique.” In this approach, the intravitreal fluid (usually balanced salt solution) is left inside the vitreous cavity, while the surgeon injects the dye. The amount of dye in contact with the retinal surface becomes much lower because it is immediately washed out by the fluid in the vitreous cavity. Czajka et al. compared the two methods in a porcine model and concluded that the air-filled technique induces a higher incidence of RPE atrophy and outer retinal degeneration than the fluid-filled technique. In conclusion, the wet method is more safer and faster during surgery than dry method.

Conclusion

Exploration of the breakthrough chromovitrectomy supports innovative microsurgical techniques with improved patient outcomes for both anterior and posterior segment procedures. Recent recommendations for the application of dyes during vitreoretinal surgery designate that ICG, IFICG, BrIβ, and Broβ may be the best stains for ILM, while TB and PB may be preferred for staining the glial ERM. In addition, the white steroid TA is an amazing staining agent for vitreous visualization. Regarding to the toxicity issues, no decided safety profiles for the different dyes in chromovitrectomy. In addition, some good words include avoidance of long macular exposure to endoillumination and, low amount of dye injection.

Conflict of interest

The authors declared that there is no conflict of interest.

References


7. IUPAC Gold Book Chromophore.


