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The Use of Vital Dyes in Ocular Surgery

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Abstract. Vital dyes have advanced diagnosis and surgical technique in various specialties, including oncology, gastroenterology, and ophthalmology. In ocular surgery vital dyes are widely used in cataract and vitreoretinal surgery. Worldwide, intra-operative use of trypan blue during cataract surgery has enhanced visualization of the anterior capsule during capsulorrhexis, and patent blue has been recently licensed in Europe for cataract surgery. For chromovitrectomy, the vital dyes indocyanine green, infracyanine green, and brilliant blue stain the internal limiting membrane, and trypan blue and triamcinolone acetonide help visualize epiretinal membranes and vitreous, respectively. Intra-operative vital dyes are finding uses in corneal, glaucoma, orbit, strabismus, and conjunctival surgery. We provide a summary of current knowledge of the use of vital dyes in ocular surgery. We review the properties of dyes, techniques of application, indications, and complications in ocular surgery. Vital dyes represent an expanding area of research, and novel dyes deserve further investigation. (**Surv Ophthalmol** 54:576–617, 2009.

Key words. vital dye • staining • retina • trypan blue • indocyanine green • vitrectomy • chromovitrectomy

Introduction

Dyes are chemical compounds that bind to various substances in nature to induce color.[178] When dyes color living tissues or cells, they are called *vital dyes*.[331] Vital dyes emerged recently as important and effective surgical adjuvants to enhance visualization of ocular tissues. In cataract surgery the blue dye trypan blue (TB) gained widespread use because it stains the anterior capsule and enables easier intra-operative removal of this fine, semi-transparent membrane.[153] In vitreoretinal surgery, greening and bluish vital dyes such as indocyanine green (ICG) and brilliant blue (BriB) also facilitated visualization and removal of pre-retinal membranes as a result of their different affinities to intraocular collagen and cellular elements.[306,309] Vital dyes has also been used in corneal, glaucoma, orbit, strabismus, and conjunctival surgery. We discuss the use of vital dyes in ocular surgery, including their biochemistry and pharmacology, their clinical effectiveness in staining ocular tissues, and the surgical techniques that utilize vital dyes, as well as clinical safety and toxicity issues.

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Biochemistry, Pharmacology, and General Information of Vital Dyes Used in Ocular Surgery

Vital staining refers to the coloration of living cells or tissues. When vital staining is done in a living organism, it may be called *intravital staining*, whereas *supravital staining* is defined as the application of dyes to living cells or tissues freshly removed from the body.[178] The various dyes currently available may be classified according to their pH, solubility, source, or staining property. A commonly used classification of vital dyes according to their chemistry is applied in this section.

AZO DYES

Azo dyes are large class of synthetic organic dyes that contain nitrogen in the azo form (-N = N-) in their molecular structures connecting aromatic ring compounds. Azo dyes give bright, high intensity colors, and their biggest advantage is cost-effectiveness. Because these can be easily chemically altered, an enormous range is available, constituting 60% of all synthetic dyes.[219]

TB is an anionic hydrophilic azo dye with the formula $C_{34}H_{24}N_6Na_4O_{14}S_4$ and a molecular weight of 960 daltons. Live cells/tissues with intact cell membranes usually are not colored because their selective control of cellular membrane transport does not allow binding of TB.[49] TB has been widely used in both vitrectomy and cataract surgery. TB is commercially available in an 0.15% concentration for vitreoretinal surgery under the brand name Membrane Blue (DORC International, Zuidland, Netherlands) and as Vision Blue in a 0.06% concentration for cataract surgery (DORC International). TB as Membrane Blue and Vision Blue comes in a solution containing small amount of sodium salts, 8.2 mg of NaCl, and water. The osmolarity of Vision Blue ranges from 257 to 314 mOsm/Kg and the pH from 7.3 to 7.6.[400,404]

Janus green (JG) is a basic azo dye of chemical formula $C_{30}H_{31}N_6Cl$ used histologically to stain mitochondria supravitally. JG changes color according to the amount of oxygen present. For ocular surgery, JG has been used in the supravital staining of corneal endothelium to assess viability before keratoplasty; however, the dye is not utilized intraoperatively.[300]

ARYLMETHANE DYES

Arylmethane dyes containin one carbon linked to two benzene or naphthalene groups bound to one moiety of N or O and one amino group.[169] The variable substitution of rings in the amino group determines further subclassification of this group of dyes, with four recognized families: diarylmethanes, aminotriarylmethanes, hydroxytriarylmethanes, and hydroxyaminotriarylmethanes.[178]

Gentian violet (GV), also known as crystal violet or methyl violet, is a water-soluble cationic aminotriarylmethane dye used mainly in histological preparations. This purple dye has a molecular formula of $C_{25}H_{30}ClN_3$ and molecular weight of 407 daltons.[178,377] GV is also used for the treatment of burns and other injuries to the skin and gums in a weak (1%) aqueous solution. In ophthalmology GV has been applied for anterior capsule visualization and as a marker of the cornea and conjunctiva.

BriB, also named Acid Blue or Coomassie, is a blue anionic aminotriarylmethane chemical compound which has the chemical formula of $C_{47}H_{48}N_3S_2O_7Na$ and a molecular weight of 854 daltons.[397] The synthetic blue agent has been certified as a food additive in Europe and may be used as a protein marker in cardiovascular and neurologic diseases.[67] Animal and human data on the use of BriB during vitreoretinal surgery and for anterior capsule staining have been described in recent years, resulting in its approval for intraocular use in Europe in 2007 under the brand name of Brilliant peel (Fluoron/Geuder, Heidelberg, Germany).[306]

Bromophenol blue (BroB), also named tetrabromophenolsulfonephthalein, is a hydroxytriarylmethane color marker dye with a molecular weight of 670 daltons and the chemical formula of $C_{19}H_{10}Br_4O_5S$. BrB has been applied in laboratorial settings as an acid-base indicator as well as marker in gel electrophoresis.[279,330] The dark-blue dye may represent a novel and useful adjunct for both cataract and vitreoretinal surgery, although no commercial available product is yet available in the United States.

Patent blue (PB) is a hydrophilic anionic triarylmethane dye with the chemical formula of $C_{27}H_{31}N_2NaO_6S_2$ and a molecular weight of 582 daltons. The blue arylmethane dye prepared in sodium or calcium salt is applied as a fluorescent indicator to identify fungi in vitro.[348] It has also found use in localizing lymph nodes during oncologic surgery.[19,46] PB has been has been certified in Europe since 2003 for capsule staining during cataract surgery in a concentration of 0.24% under the brand name of Blueron (Geuder) and has been applied off-label during vitreoretinal surgery.[124]

CYANINE DYES

Cyanine dyes contain a -CH = group linking two heterocyclic rings containing nitrogen. Cyanine dyes are part of a larger group called polymethine dyes. Cyanine dyes are highly colored, organic com-

pounds first synthesized over a century ago. They have been mostly used as sensitizers in photography or textile dyeing.[62]

ICG is a tricarbocyanine anionic vital dye with a molecular formula of $C_{43}H_{47}N_2NaO_6S_2$ and mass of 775 daltons.[212] The cyanine agent has amphiphilic properties that allow it to bind to both cellular and acellular elements in living tissues.[62,63] The hydrophilic dye is delivered as a sterile powder, and in 1959 it was approved by the FDA for indicator dilution studies and for liver function testing. Both the absorption and fluorescence maximums of ICG are within the near-infrared range. ICG represents is useful in retinal angiography, because it improves visualization of choroidal tissues. In ocular surgery its use remains off-label despite widespread popularity.[5,37] ICG is commercially available under the names of ICG-Pulsion (Pulsion Medical Systems; Munich, Germany; 25 mg and 50 mg vials); ICV Indocianina Verde (Ophthalmos, São Paulo, Brazil; 5, 25, and 50 mg vials); Diagnogreen (Daiichi Pharmaceutical, Tokyo, Japan; 25 mg vial); and IC-Green (Akorn Inc, Buffalo Grove, IL, USA; 25 mg vial). ICG commercial products contain from 4–5% iodine, which represents both residues of the dye synthesis process and the iodine in the molecular formula. The dye is commercially provided as a powder to achieve final concentrations of 0.05% to 0.5%.[306,309] It is recommended that the green dye initially be diluted in distilled water before further dilution in saline solution, because of a higher risk of precipitation in saline. One experiment demonstrated precipitation when diluted with BSS Plus, but not with physiological saline solution.[267]

Infracyanine green (IfCG) is a green dye with the same chemical formula and similar pharmacologic properties as ICG. IfCG is commercially available under the brand name of Infracyanine (Laboratoires SERB, Paris, France; 25 mg vial). IfCG dye possesses two pharmacologic differences when compared to ICG. First, IfCG contains no sodium iodine, which must be added to ICG during the dye synthesis. Second, the presence of the sodium iodine in the ICG solution necessitates dilution in water, resulting in a hypotonic solution of 248 to 275 mmol/kg. The iodine-free IfCG is usually dissolved in 5% glucose , generating an iso-osmotic solution of 294 –314 mmol/kg. IfCG represents a desirable contrast agent for visualizing pre-retinal membranes and tissues, because it spares the retina exposure to iodine and its derivatives.[186]

THIAZINE DYES

In chemistry thiazines are organic chemical compounds with one ring of four carbon, one nitrogen, and one sulfur atom that may generate various molecules that act as dyes, tranquilizers, and insecticides. The thiazine dyes used in biology and medicine are always a small conjugated system, mostly cationic.[383]

Methylene blue (MB) is a heterocyclic aromatic dye with molecular formula of $C_{16}H_{18}ClN_3S$ and a molecular weight of 319 daltons. MB is familiar as a component of Gram stain.[50] MB has been injected or sprayed intra-operatively as an adjunct in colonoscopy, endoscopy, or for sentinel lymph node dissections.[220] MB has been recently used in ophthalmology for guiding layered excision of certain cutaneous carcinomas, for the removal of orbit dermoid cysts, and to facilitate identification of the adipose pocket during blepharoplasties.[18]

Toluidine blue (ToB) is a metachromatic blue dye of chemical formula $C_{15}H_{16}N_3SCl$ and a weight of 305 daltons that is used frequently for histologic staining. Clinically this thiazine compound has been useful in early detection of suspicious lesions in the mouth and oropharynx, while in chromoendoscopy ToB may localize squamous cell carcinoma of the esophagus.[179] In ophthalmic surgeries the blue thyazine compound ToB is used to stain conjunctival tumors, while toxicity limits its application intraocularly.[160]

XANTHENE DYES

The term xanthene is applied to a yellow organic heterocyclic compound of chemical formula is $C_{13}H_{10}O$. Xanthene is a source of an interesting class of dyes named the xanthene dyes. Fluorescein, eosin, and rhodamine are derived from xanthene. Xanthene dyes fluoresce yellow to pink or bluish to red. This class of dyes is divided into various subgroups based on ionicity or lipophilicity/hydrophilicity.[178]

Fluorescein is a xanthene fluorophore with chemical structure of $C_{20}H_{12}O_5$ and molecular weight of 332 daltons. The vital dye in water has a very high fluorescence with an absorption maximum and excitation at 494 nm and emission maximum of 521 nm. Fluorescein may be conjugated with over 50 different salts or derivatives, including fluorescein sodium (FS) and fluorescein diacetate (FD). Fluorescein derivates have been commonly used in microscopy, in dye laser as the gain medium, in serology to detect latent blood stains, and in dye tracing. Fluorescein is used extensively as a diagnostic tool, mainly as FS. In ocular surgery the xanthene compound has been shown to stain the vitreous gel either in the form of FS or FD.[69,106,157]

NATURAL STAINS

Alizarin red (AR) is a red dye that was isolated initially from the root of madder plants, but by the

end of 19th century was produced synthetically. The red pigment has molecular formula $C_{14}H_8O_4$ and molecular mass of 240 daltons. AR has been applied in biochemical assays to assess by colorimetry the presence of calcium deposition by cells of an osteogenic lineage, while clinically AR colors calcium phosphate crystals in synovial fluid.[107] In the laboratory AR was found to stain denuded Descemet's membrane (DM) and to delineate viable and non-viable cells borders in corneas,[362] but has not been used clinically.

STEROIDS

Corticosteroids are a group of hormones naturally produced in the adrenal cortex. Synthetic drugs with corticosteroid-like effect are familiar to clinicians and include dexamethasone, triamcinolone, and fluorometholone. Triamcinolone acetonide (TA) is a synthetic insoluble corticosteroid of empirical formula $C_{24}H_{31}FO_6$ and molecular weight of 434 daltons, whereas fluorometholone acetate (FMA) is a synthetic fluorinated glucocorticosteroid with a chemical formula of $C_{24}H_{31}FO_5$ and a molecular weight of 418 daltons.[122] Synthetic corticosteroids have been used in the treatment of various edematous and inflammatory vitreoretinal diseases such as macular edema. In ocular surgery both TA and FMA stain the vitreous, mainly because of crystal deposition.[154,306,308] Triamcinolone acetonide is commercially available under the brand names of Triesence (Alcon Labs, Forth Worth, TX, 40 mg/ml); Kenalog (Bristol-Myers-Squibb, Peapack, NJ, 40 mg/ml); Trivaris (Allergan, Irvine, CA, 80 mg/ml); and Triancinolona (Ophthalmos Ind., 40 mg/ml).

The Use of Vital Dyes in Corneal Surgery

RATIONALE

Vital dyes have been used to assess endothelial cell viability for many years.[352,357,380] More recently, the development of novel keratoplasty techniques broadened the intra-operative usefulness of vital dyes in corneal surgery. Intra-operative staining of the cornea may allow better visualization of the endothelial cells, corneal incisions, corneal stroma, or DM, thereby enhancing final surgical outcomes.

VITAL DYES FOR CORNEAL ENDOTHELIAL CELLS VIABILITY

Storage of human corneas before transplantation leads to endothelial cell death, thereby reducing endothelial cells density.[241] Corneal banks assess the viability of the corneal endothelium by various techniques: measuring cell density using specular microscopy, optical microscopy, or vital dye staining.[339] The main advantage of the dye-guided technique is the simple and fast detection of non-viable cells. On the other hand, vital dye techniques may underestimate the number of non-viable cells because some impaired cells with intact membranes are not stained.[86]

Trypan Blue (TB)

TB has been used in many eye banks to evaluate corneal endothelial cell viability.[380] The blue dye stains the nuclei of damaged and dead endothelial cells in donor corneas, as well as areas of DM denuded of endothelial cells.[352,357] In order to assess endothelial damage and endothelial cell density by light microscopy, Sperling in 1986 introduced the use of TB diluted in 0.45% and 0.9% sodium chloride, or with 1.8% sucrose to induce dilation of intercellular spaces for visualization of endothelial cell borders.[352] However, the reliability of TB-guided endothelial cell counts has been questioned.[86] Although there was a significant correlation between the techniques, TUNEL-assay seemed to be more reliable in detecting earlier events in endothelial death process. At the frequently used concentrations of 0.001–0.1%, TB was not toxic to the corneal endothelial cells.[380] Further investigation may clarify the benefits of TB-guided endothelial cell assessment for clinical practice.

Janus Green (JG)

JG has been used for decades as indicator of cell damage in corneal tissue for assessing endothelial cell viability.[121,296] JG has similar staining properties as TB, binding to the disrupted cellular membrane. An in vitro study demonstrated a positive correlation between this technique and microscopic cell counting. When a large number of cells had been lost, assessment with JG was better because of the difficulty of the microscopic cell counting technique.[296] JG staining of the corneal endothelium may be an indicator of cellular membrane integrity, a parameter of cell damage, but has not yet been widely used.

Indocyanine Green (ICG)

The use of ICG for assessment of endothelial cell viability was initially proposed in 1978.[240] In that work the authors showed that ICG did not damage living cells and almost exclusively stained dead cells. More recently, the safety of ICG on corneal endothelial function, ultrastructure, or viability has been demonstrated.[134] The green dye did not compromise cell integrity, as determined by electron microscopy, when rabbit and human corneal endothelia were exposed to 0.5% ICG for 3 minutes. Although it has

the same indication as TB, ICG staining for endothelial cell count has not gained popularity.

Alizarin Red (AR)

AR is an intercellular dye that stains denuded DM and may be useful to delineate viable and non-viable cells borders, being an adjunct to TB in a technique called the *dual staining procedure*.[362] Its use has been limited to laboratory studies.[142]

Fluorescein Diacetate

This vital dye stains non-viable corneal endothelial cells.[400] Its uptake and metabolism by these cells is an active process, and duration of staining is onlyabout 40 minutes.[399] Therefore, care should be taken to avoid false-negative results. Although showing promise, it has not replaced TB for the identification of impaired endothelial cells in donor corneas.

VITAL DYES FOR KERATOPLASTY

New techniques for corneal transplantation surgery, such as anterior lamellar keratoplasty, deep lamellar endothelial keratoplasty (DLEK), or Descemet's stripping endothelial keratoplasty, are leading to better surgical outcomes, although these techniques reauire accurate identification of the layers of the cornea. Vital dyes may improve identification of these layers by staining specific structures such as the collagen fibers of the stroma, endothelial cells, and DM in both the donor and host corneas. Different dyes have been used for specific steps in the various keratoplasty techniques, but there is as of yet no standardization.[16]

Trypan Blue

TB is used in both penetrating and deep lamellar keratoplasties.[16,311] For penetrating keratoplasty, 0.02% TB solution may be injected into the anterior chamber via a paracentesis to stain the DM of the donor as well as the recipient corneal tissue. Dye exposure may promote close alignment of edges of host and donor DM, thus improving graft stability and minimizing surgically induced astigmatism. TB may show both cut edges well, identify the corneal depth during suturing; and enable visibility of the viscosurgical device removal. Thereby the blue dye may improve tissue apposition.[311]

TB has been also used in order to facilitate stromal dissection and to avoid DM perforation in lamellar keratoplasty.[16] After a two-thirds trephination of the cornea, 0.02% TB solution is injected intrastromally in four quadrants through a 30-gauge cannula for superficial dissection. Next, more TB is injected for deep dissection to stain the stromal fibers. This novel approach resulted in no residual stroma at the end of the intervention. TB disappeared totally in the early postoperative period.

Finally, retained DM after penetrating keratoplasty is a common complication in congenital hereditary endothelial dystrophy because of longstanding stromal edema and loosening of the attachment. The surgical removal of these retained membranes after application of 0.1% TB has been reported. In this case, the simple, quick application of TB facilitates the removal of retained membranes during penetrating keratoplasy.[342] In summary, the TB stain may be useful intra-operatively to visualize and remove the posterior stromal layers in modern keratoplasty.

Indocyanine Green

ICG has been reported as an useful agent for DLEK.[156] After dissection and excision of the host posterior stromal disk, including DM and endothelial cells, ICG is used to stain corneal stroma of the donor disk transplanted to the host anterior chamber. After being placed by an air bubble, ICG staining allowed visualization of the tissue interface through the host corneal stroma. After 24 hours the ICG disappeared from the cornea, with no signs of inflammation. The authors conclude that ICG safely facilitated the DLEK procedure.

Gentian Violet (GV)

GV is proposed to mark the peripheral stromal surface containing DM and endothelium in cases of DLEK, Descemet's stripping, and automated endothelial keratoplasty.[187] By identifying the side of the marked cornea, surgeons may insert the folded donor disk into the host anterior chamber in the proper position. This ensures that air is injected posterior to the graft, thus preventing the donor lenticule from unfolding upside down.

VITAL DYES FOR CLEAR CORNEAL INCISION WITH COATED BLADE

Trypan Blue

Due to the frequent difficulty in finding clear corneal incisions during intraocular surgery, the use of TB-coated blades has been proposed.[170] A 3.0-mm phaco incision blade tip was coated with TB in order to improve visualization. Fluid outflow and the mechanical effect of phaco tip movement during surgery also facilitated visualization of the incision, and no complications were reported with this surgical method. The vital dye had disappeared from the cornea after few days.

The Use of Vital Dyes in Conjunctival Surgery

RATIONALE

Inflammatory and neoplastic conjunctival lesions often manifest as semi-transparent structures, which in some instances may be difficult to differentiate from normal tissue. Squamous cell carcinoma (SCC) of the conjunctiva is managed according to the extent and depth of the lesion. In order to perform surgery for removal of an SCC of the conjunctiva and completely reconstruct the remaining conjunctiva successfully, surgeons should locate the margins of the conjunctival lesion.[338] In addition, benign lesions such as pterygium or conjunctival cysts may infiltrate surrounding healthy conjunctiva. Staining neoplastic masses and benign inflammatory or congenital lesions with a vital dye facilitates their complete intra-operative removal, and also aids in successful reconstruction of the remaining conjunctiva.[160]

VITAL DYES FOR IDENTIFICATION OF CONJUNCTIVAL TUMORS

The biological stain ToB has been used as intra-operative adjunct in conjunctival surgery. With the aid of the ToB, conjunctival tumors can be precisely removed en bloc with reconstruction of the remaining conjunctiva, leaving the border of the excised surgical specimen negative for carcinoma cells.[160] Furthermore, pterygia, pingueculae, papilla, follicles, and dry eyes do not stain with ToB, and vital staining with ToB can be used to detect malignant lesions. In conclusion, vital staining of the conjunctiva with ToB is effective in assisting in the detection of the precise location of the SCC, making complete removal easier and aiding in successful reconstruction of the remaining conjunctiva.

VITAL DYES FOR IDENTIFICATION OF CONJUNCTIVAL CYSTS AND PTERYGIUM

Inclusion cysts of the conjunctiva can be congenital or acquired. Acquired inclusion cysts form as result of implantation of conjunctival epithelium underneath the stroma following injury or ocular surgery. Simple resection is usually curative; however, incomplete resection results in recurrence of the lesion. Identification of the margin of the cyst is sometimes difficult, and fragility of the cyst capsule also makes complete removal difficult.[338]

The vital dyes ICG and TB aid in visualization of conjunctival cysts capsules.[183,185] ICG can be injected through a 27-gauge needle into the conjunctival cyst. Following the flushing of residual ICG solution, the cyst becomes clearly visible as a green-stained structure. With use of ICG alone, however, the cyst wall may collapse during injection of the dye, resulting in insufficient staining and difficulty in separating cyst from conjunctiva. Kobayashi and Sugiyama reported a modified method using a mixture containing 2.3% sodium hyaluronate and 0.06% TB solution.[184,185] The high viscosity combination distends the capsule and prevents it from collapsing during cyst removal. Such techniques may also have a role in facilitating visualization and excision of other cystic lesions from the ocular surface.

The Use of Vital Dyes as Adjuvant in Cataract Surgery

RATIONALE

The continuous curvilinear capsulorrexis (CCC) is a critical step in phacoemulsification. Adequate red reflex is important for visualizing the anterior capsule and maintaining control of the capsule tear. In situations in which the red reflex is compromised—such as white cataracts, traumatic cataracts, corneal opacities, or posterior segments alterations—the CCC may be a particular challenge. To address this problem, staining of the anterior capsule was initially reported in 1993.[133] Since then, the use of vital dyes as an adjuvant in cataract surgery has been widely reported.[99,172,341]

VITAL DYES AS SURGICAL ADJUVANT FOR ANTERIOR CAPSULE IDENTIFICATION

Trypan Blue

TB staining of the capsule edge in CCC was initially introduced in 1999.[244,245] Since then the blue azo dye has been the most frequently used agent for staining the anterior capsule, as intra-operative injection of TB provides various advantages in cataract surgery (Fig. 1). First, surgical TB injection may promote higher rates of success of capsulorrhexis in phacoemulsification for cases with inadequate red reflexes.[190,268,280,364,366] The rate of conversion to an extracapsular cataract extraction in white cataracts as the result of an incomplete CCC has been as low as 3.85% when TB is used, compared to 28.3% when no dye has been injected.[58,152,268,281,312] Second, the blue vital stain allows staining of anterior and posterior capsules in children younger than 5, thereby enhancing effectiveness for completing the CCC.[31,177,282,283,316] Thirdly, the importance of TB in the learning process of trainee surgeons and residents to perform CCC has been recently highlighted.[57,396] Even in the presence of a good red reflex, young surgeons had a higher success rate of CCC when the dye was used.[396] In a series of 11 cases with corneal opacities, the CCC was completed in all instances in

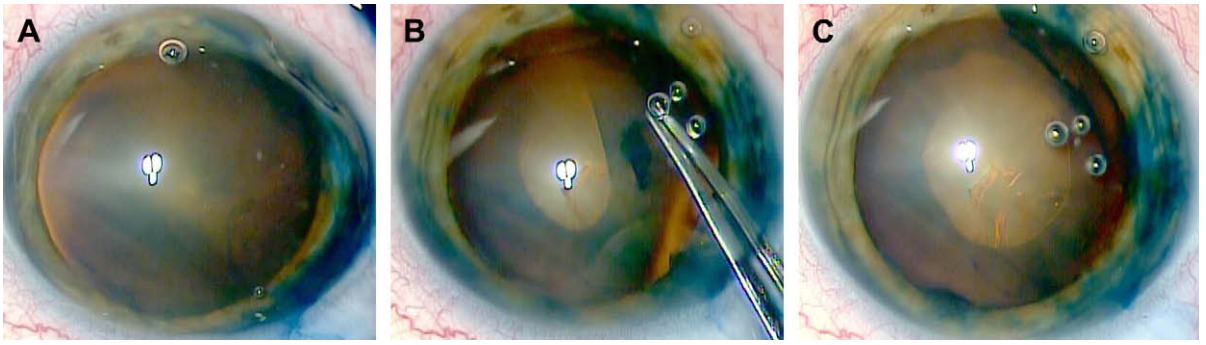

Fig. 1. Intra-operative anterior capsule staining with trypan blue in cataract surgery. *A:* Intracamerular injection of 0.06% trypan blue stains the anterior capsule greatly in a dark-blueish color. *B:* The surgeon may identify the edge of the capsulorrhexis as the blue anterior capsule is contrasted to the uncolored lens cortex and nucleus. *C:* At the end of the curvilinear capsulorrhexis maneuver, further steps may be executed during the emulsification surgery.

which TB was used.[22] Finally, 0.3 ml of 0.06% TB has been applied to find the edge of a lost capsulorrhexis, allowing the CCC completion in all cases.[381]

One hypothesis to explain the positive effect of TB staining of the lens capsule is that it modifies of the biomechanical structure. Wollensak and coauthors recently performed a study on porcine eyes that evaluated 0.1% TB stain for 30 seconds, 1 minute, and 30 minutes with or without white light.[401] TB staining for 1 and 30 minutes led to an increase in elastic stiffness and a significant decrease in ultimate extensibility in the anterior capsule. This effect was probably due to the photosensitizing action of TB to physical cross-linking of collagen through yielding of free oxygen radicals, which causes a change in elastic behavior.[323]

Recently, Singh et al analyzed histological characteristics of TB-stained human capsules.[340] They found out that TB mostly stained the basement membrane adjacent to the epithelial layer of the lens capsule with minimal laminar staining in the superficial basement membrane. Such findings may also explain why TB may provide differentiation between non-stained cortex and the lens capsule.[297]

Studies regarding the efficiency of different concentrations of TB in staining the anterior capsule, comparing concentrations ranging from 0.0125% to 0.5%, have been performed.[24,316,387,413] Yetik et al diluted 0.1% TB in balanced saline solution, producing four different concentrations (0.05%, 0.025%, 0.0125%, and 0.00625%).[413] These solutions were used in 45 eyes of 35 patients. The researchers injected 0.1 ml of dye with either the classic air-bubble technique or with the viscoelastic technique. Surgeon evaluations show that concentrations as low as 0.0125% stained the anterior capsule satisfactorily. Most clinical studies prefer 0.1% TB to stain the anterior capsule, even though very effective staining has been achieved with concentrations as low as 0.06% (Table 1A).[40]

Effective staining in cataract surgery also depends on the exposure time. An in vitro study by Fritz, with human anterior capsules excised during standard capsulorhexis, found that 17% color saturation was considered as a good stain by the surgeon.[82] In their study TB 0.1% stain was evaluated with 30 seconds, 60 seconds, 5 minutes, 30 minutes, 6 hours, and 24 hours. 17% saturation was reached after 1 minute of dye exposure. The minimum incubation time reported for successful capsule staining ranges from 5 seconds to 2 minutes (see Table 1A).[316,404]

Indocyanine Green

ICG staining for capsule visualization in conditions with poor or no red reflex has been reported since the end of the 1990s, using concentrations ranging from 0.125% to 0.5%.[105,135,136,175,265] According to an in vivo study in rabbit eyes, the lowest effective concentration of ICG to provide a comparable contrast with TB to the anterior capsule was 0.25%.[38,40]

Comparative studies about staining effectiveness between TB and ICG for CCC has led to controversy. Xiao et al in their in vivo analysis found that TB under an air bubble was a slightly superior coloring agent, although the success rate for achieving CCC in either groups of ICG or TB dye was not significantly different.[406] On the other hand, Pandey et al, in a study with post-mortem human eyes, concluded that intracameral subcapsular injection of ICG was superior to TB in enabling anterior CCC visualization.[281] In these two mentioned studies, different techniques were applied, which may explain such opposing results. One advantage of TB over ICG is lower cost.

Fluorescein Sodium

FS in a 2% concentration was the first dye used to stain the anterior capsule, in 1993.[101,133] In the

TABLE 1A

Staining the Anterior Capsule: Clinical Humans Studies

Author et al (publication year)	Study Design	Level of Evidence	Dye Concentration (%)	Incubation Time (sec)	Injection Technique	Study Purpose	Intraocular Toxicity
Hoffer (1993)[133]	Case series	III	FS 2	NA	Subcapsular	Describe new technique	Not present
Fritz (1998)[83]	Case series	III	FS 2	NA	Under air bubble	CCC in hipermature cataracts	Not present
Horiguchi (1998)[135]	Experimental	II	ICG 0.5	NA	Under air bubble	CCC in hipermature cataracts	Solutions above 0.25 were toxic to cornea endothelia
Melles (1999)[245]	Experimental	III	TB 0.1	NA	Under air bubble	Evaluate a new dye	NA
Pandey (2000)[281–283] Part 1, 2 and 3	Experimental (postmortem human eyes)	III	TB 0.1 ICG 0.5 FS 2	NA	Under air bubble and intracameral subcapsular	Evaluate of anterior and posterior capsule stain	NA
Newsom (2000)[265]	Case series	III	ICG 0.5	NA	Under air bubble	Dye was used in a anterior capsule rupture	Not present
Kayikicioglu (2001)[171]	Experimental	III	TB 0.4	NA	Mixed with viscoelastic	Describe new technique	NA
Kothari (2001)[190]	Prospective	III	TB 0.1	5 to 10	Under air bubble	Evaluate anterior capsule stain	NA
Jacobs (2002)[152]	Prospective	III	TB 0.06	NA	Under air bubble	Evaluate anterior capsule stain	NA
Bhartiya (2002)[22]	Prospective	III	TB 0.1	10	Under air bubble	CCC in corneal opacities	Not present
Van Dooren (2002)[381]	Prospective	II	TB 0.06	NA	Under air bubble	One-year follow-up to evaluate corneal damage	7.5% endothelial loss in stained cases against 10.2% in control group
Wakabayashi (2002)[387]	Case series	III	ICG 0.5	300	Under air bubble	PCC in congenital cataracts	NA
Fritz (2002)[82]	Experimental	III	TB 0.1 FS 2	NA	Under air bubble	Intensity of stain in anterior capsules and various IOL materials	NA
Yetik (2002)[413]	Experimental	III	TB 0.00625; 0.0125; 0.025; 0.05	NA	Under air bubble and under viscoelastic	Determining the lowest effective concentration	NA
Werner (2002)[395]	Case series	III	TB 0.1	NA	Under air bubble	Effects on hydrogel IOL	NA

(*continued on next page*)

Table 1A (*continued*)

Author et al (publication year)	Study Design	Level of Evidence	Dye Concentration (%)	Incubation Time (sec)	Injection Technique	Study Purpose	Intraocular Toxicity
Khokhar (2003)[176]	Experimental	III	TB 0.06 ICG 0.5	NA	Under viscoelastic	New painting cannula	NA
Horiguchi (2003)[136]	Experimental	II	ICG 0.125	10 to 30	Under viscoelastic	Kinetics of ICG after capsule staining	NA
Saini (2003)[316]	Prospective	II	TB 0.1	30 to 40	Under air bubble	Efficacy of TB to create a CCC and a PCC	NA
Singh (2003)[340]	Experimental	III	TB 0.06	NA	Under air bubble	Histological analysis of lens capsules	NA
Burk (2003)[33]	Case series	III	TA 0.4; 4	NA	NA	Describe anterior vitreous stain technique	NA
Bisol (2004)[24]	Prospective	II	TB 0.1	NA	NA	Effect of TB staining of hydrophilic IOL on contrast sensitivity and glare	TB 0.1% for 30 min was toxic to corneal fibroblasts
Dada (2004)[59]	Prospective	III	TB 0.1; GV 0.001; ICG 0.5; FS 2 and autologous blood	NA	Under air bubble	Compare safety and efficacy of different dyes	All dyes were safe
Laureano (2004)[214]	Experimental	III	TB 0.1	30	One-step technique	Describe a new technique	NA
Xiao (2004)[406]	Prospective	III	TB 0.1 ICG 0.5	NA	Under air bubble	Efficacy and safety	Not present
Chung (2005)[47]	Prospective	II	TB 0.1 ICG 0.5	120	Under air bubble	Safety of TB and ICG	No endothelial damage was found
Allen (2006)[4]	Case report	III	ICG 0.5	NA	Under air bubble	Traumatic cataract and vitreous leakage of the dye	Increase of the IOP and decrease of visual acuity
Brouzas (2006)[30]	Case report	III	MB 1	NA	Under air bubble	Inadvertent anterior capsule stain	Severe corneal edema
Cacciatori (2006)[35]	Case series	III	TB 0.06	60	Under air bubble	TB to highlight vitreous in anterior chamber	NA
Jacobs (2006)[153]	Review	NA	NA	NA	NA	Review of dyes used to stain lens capsule	NA
Nanavaty (2006)[257]	Prospective	II	TB 0.0125	30	Under air bubble	Evaluate lens epithelial cells toxicity	Decrease in density and viability of lens epithelial cells
Wong (2006)[404]	Prospective	III	TB 0.06	5	Under air bubble and under viscoelastic	Compare different techniques	Not present

initial report of FS application during cataract surgery, Hoffer and McFarland described injection of the coloring agent underneath the capsule.[133] Later on, Fritz improved the technique by using blue light during CCC to visualize staining of the epithelium located on the inner surface of the capsule.[83] This novel approach enabled the surgeon to identify the tearing edge accurately, ensuring a continuous circular opening. In the same study, the author also mentioned some disadvantages of FS, such as corneal staining and vitreous leakage, which may occurred due to its low molecular weight.

Few clinical investigations analyze the staining properties of different concentrations of FS in cataract surgery. Chang et al evaluated the efficacy of FS applied under an air bubble in rabbit eyes and found 1.25% to be the lowest concentration that stained satisfactorily.[40] Fritz analyzed the time of exposure of 2% FS in excised human anterior capsules.[82] After only 5 minutes 2% FS reached 17% saturation, providing good staining while retaining some translucency. However, the lack of contrast against white lenses makes FS use difficult, suggesting that a satisfactory stain with a shorter exposure time would be better achieved in a subcapsular application technique. The migration of FS into the vitreous cavity and staining of the lens cortex and nucleus of the and corneal endothelium result in inadequate contrast between the lens capsule and cortex , limiting FS's usefulness as an aid in performing capsulohrexis.

Gentian Violet

Anterior capsule staining with GV in humans was first presented in 1998 (XVIth Congress of the European Society of Cataract and Refractive Surgeons, Nice, France, September 1998). Since then, only a few papers have addressed the use of this dye in cataract surgery. Unlu et al compared two concentrations of GV, 0.01% and 0.001%, in terms of obtaining a satisfactory stain, of the success rate of CCC, and of their intra-operative and post-operative complications.[377] They found no difference between the two concentrations, except a surgeon's subjective impression that a better stain is achieved with 0.01%.

Brilliant Blue (BriB)

A recent study of BriB in enucleated pig's eyes raises the possibility of a new dye for cataract surgery.[129] Concentrations varying from 10 to 0.01 mg/ml were tested. The minimal concentration needed to produce high-quality staining was 0.025% after immediate wash-out of the dye. Further studies should elucidate the role and safety of BriB (Fig. 2).

SURGICAL TECHNIQUE FOR STAINING THE ANTERIOR CAPSULE IN CONTINUOUS CURVILINEAR ANTERIOR CAPSULORREXIS

Various techniques have been proposed with the objectives of facilitating the CCC, minimizing endothelial damage, reducing surgery time, establishing the lowest dye concentration for an effective staining, and identifying the vitreous in cases of capsule rupture. These are: 1) air bubble injection, 2) intracameral subcapsular injection, 3) injection under viscoelastic agents, and 4) intracamerular one-step injection.

Air Bubble Injection

Melles and co-authors filled the anterior chamber with air through a corneal incision and injected the vital dye through a cannula under the air bubble for 30 seconds.[244,245] Benefits of this technique include better staining of the peripheral anterior capsule rim, as well as lack of dye contact with corneal endothelium. However, air can easily escape from anterior chamber, thereby raising the lens–iris plane. A small amount of high-density viscoelastic material placed near the incision may prevent air bubbles from exiting the anterior chamber. Fainter staining with the air bubble technique may be explained by a progressive dilution by aqueous remaining in the anterior chamber.

Intracameral Subcapsular Injection

In the intracameral subcapsular injection, aqueous is replaced with viscoelastic, and the dye is carefully injected beneath the anterior capsule with a small-gauge needle. The dye remains trapped in the subcapsular space, which enables the staining agent to remain in contact with the posterior surface of the anterior capsule, especially in the center and midperiphery. Despite the fact that both capsule and cortex are stained by the dye, they can be clearly distinguished by the feathery appearance of the cortex and the smooth staining of the capsule. The anterior capsule may tear if excessive dye is injected. This technique was originally proposed for FS with blue-light enhancement, but it can be also used with ICG, TB, and FS without any special illumination.[133,282,283]

In post-mortem human eyes, Pandey and co-authors evaluated anterior capsule staining using TB, ICG, and FS with both air bubble and intracameral subcapsular injection techniques. They concluded that both techniques promoted visualization of the anterior capsule, however, an intracameral subcapsular injection of ICG was slightly superior to the other dyes.[282,283]

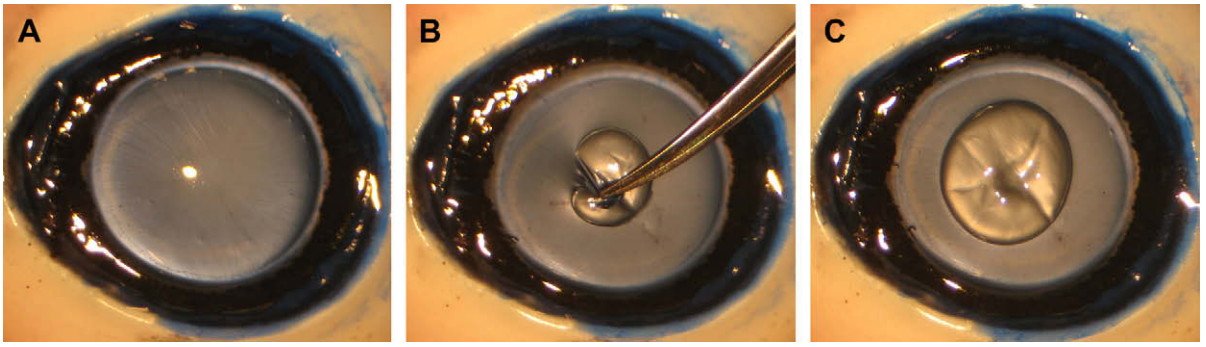

Fig. 2. Anterior capsule staining with brilliant blue in porcine eyes. *A:* Intracamerular injection of 0.5% brilliant blue may color the anterior capsule. *B:* The capsulorrhexis may be performed after the purple-blueish stained capsule contrasted with the underlying unstained lens material. *C:* The fast and successful capsulorrhexis enables the following steps of the cataract surgery.

Injection Under Viscoelastic Agents

Injection of vital dyes under viscoelastics remains an alternative for CCC staining in order to minimize endothelial damage. The modified technique involves placement of a drop of the dye onto the anterior capsule under a viscoelastic agent, such as methylcellulose or sodium hyaluronate. Next, the anterior chamber is thoroughly irrigated, and fresh viscoelastic material is again injected. Disadvantages of injection under viscoelastics agents may include poor control of the dye diffusion, peripheral dissection of the dye through the zonules into the vitreous, higher cost, and in loss of red reflex.[136,176,404,413]

Several variations have been reported. In one method, 0.4% TB is mixed with 1% sodium hyaluronate in a 1:1 ratio to achieve a homogeneous-colored solution.[171] Air is injected into the anterior chamber. The colored solution containing TB in sodium hyaluronate is then injected onto the anterior lens capsule, either under air or under a high-viscosity viscoelastic shell. Thereafter, the staining solution is completely washed out, and the anterior chamber refilled with a clear viscoelastic agent. In another variant, a special painting cannula designed by Khokhar is used under a viscoelastic agent. In this method, it may not be necessary to inject new viscoelastic material, thereby decreasing the surgical time.[176]

Wong et al prospectively compared staining under an air bubble and under viscoelastic.[404] Using an air bubble had the advantage of creating a "dry lake" over the lens capsule and being less expensive; however, it is more difficult to maintain anterior chamber depth, and the dye is more likely to leak out. With injection under viscoelastic, it is easier to maintain the anterior chamber, and the contact of dye with corneal endothelium is minimized. Wong et al found both techniques were equally effective and safe to cornea endothelia, concluding that the choice of procedure should rest on the surgeon's preference.

Intracameral One-step Injection

In the one-step method for staining the anterior lens capsule, the vital dye is instilled via a paracentesis port at the beginning of cataract surgery.[214] Aqueous humor is allowed to exit the anterior chamber, which shallows, and the resulting pupil block confines the coloring agent to the anterior chamber. An ophthalmic viscosurgical device is used to flush dye-stained aqueous from the anterior chamber, circumventing the need for anterior chamber washout. Although the device may be tinged with dye, this may not affect the CCC. This method has the advantage that it requires no additional instruments or materials and is faster.

POSTERIOR CAPSULORRHEXIS

Post-operative posterior capsule opacification may be particularly high in pediatric patients. A primary posterior capsulorhexis, therefore, improves the likelihood of a clear visual axis post-operataively.[282,283]

In a prospective, randomized clinical study, Saini et al evaluated the use of 0.1% TB for posterior CCC in 42 cataract surgeries in children under 5.[316] The dye was placed under an air bubble after the lens material had been completely removed. When no dye was used, the posterior CCC was completed in 53.8% of eyes, compared to 88.8% of those injected with TB. Wakabayashi et al used ICG in two cases of congenital cataracts and completed CCC in both without complication.[387] Further studies involving effective concentration and long-term side effects of TB and ICG in performing posterior CCC for pediatric cataracts are needed.

VITAL DYES FOR VISUALIZATION OF THE ANTERIOR VITREOUS IN CATARACT SURGERY

Vitreous loss during cataract surgery may result in complications including dropped lens fragment, retinal detachment, and cystoid macular edema.

The risk of these complications is reduced by a meticulous vitreous clean-up. In order to facilitate anterior vitrectomy, the dye-enhancement technique was initially described by Chang et al in 2006.[39]

Triamcinolone Acetonide

Burk and co-authors created a model of vitreous prolapse using cadaver eyes through a zonular dialysis and then injected undiluted TA into the anterior chamber. The TA suspension spread throughout the prolapsed vitreous. Next, they reported a series of three cases with zonular dialysis in which the injection of TA was used to visualize the vitreous with no post-operative complications.[33] Yamakiri et al injected 0.5 ml of TA following rupture of the posterior capsule in six patients. The TA stained the transparent vitreous and was easily removed with a vitreous cutter intra-operatively.[408] TA functions like a dye intra-operatively to identify prolapsed vitreous.

Trypan Blue

0.06% TB was injected into the anterior chamber of three patients with vitreous when a prolapse.[35] The dye was placed under an air bubble, and after 1 minute it was removed with the vitreous cutter. No residual staining was visualized, and no side effects were observed. Nevertheless, further studies may be needed to establish the safety and efficacy of the TB for the purpose of vitreous removal.

COMPLICATIONS AND ANTERIOR SEGMENT TOXICITY

Trypan Blue

Low doses of TB do not produce inflammation and corneal toxicity when injected into the anterior chamber.[23] Clinically, TB to facilitate CCC is used in concentrations from 0.0125% to 0.4%.[171,257] One uncontrolled case series where 0.1% TB was instilled into the anterior chamber found no effect on endothelial cell count or pachymetry during 8 years of follow-up.[269] Another randomized clinical trial found 7.5% endothelial cell loss when 0.06% TB was placed under air bubble, compared to the 10.2% loss in the group with only air.[381] In a rabbit model TB showed no corneal endothelial cytotoxicity after 1 minute exposure even at a concentration of 0.4%.[38,40] TB placed under an air bubble or under viscolelastic produced no toxicity in the follow-up period.[404] TB staining of the anterior capsule may not result in corneal toxicity (Table 1B).

Density and viability of lens epithelial cells (LEC) predicts posterior capsule opacification after cataract surgery. Nanavaty et al used 0.0125% TB to perform CCC in patients with white cataracts, and a sample of the anterior capsule was excised and compared with non-stained capsules.[257] A significant decrease in LEC density was detected in eyes treated with TB compared with untreated ones, suggesting a toxic effect to the LEC. On the other hand, Melendez et al found neither toxicity nor photosensitization in cultured LEC with concentrations of TB ranging from 0.025 to 4 mg/ml.[239] Further studies are needed.

A relative contraindication for TB is the use of hydrophilic expandable acrylic intraocular lenses (IOLs), which have the highest water content (73.5%) of currently manufactured implants. This IOL is implanted dry, and its expansion depends on hydration by fluids in the capsular bag. Because the TB that remains in the aqueous humor may be absorbed into the lens, patients may experience more glare than with other IOLs; however, no significant difference was found in contrast sensitivity.[24]

Bascal et al report a case where an area of zonulysis allowed 0.06% TB to enter the vitreous cavity.[12] They observed a moderate bluish hue of the vitreous that persisted until the fourth day. Multifocal ERG showed reduced responses which normalized after one month from the surgery, indicating a transitory retinal toxicity.

Indocyanine Green

There is little evidence showing that ICG is harmful to anterior segment structures: however, long term effects have not been fully evaluated. Chung and co-authors evaluated the mean loss of endothelial cells in phacoemulsification after a 3-month follow-up.[47] No post-operative complications have been found, and the endothelial loss showed no statistical difference when 0.5% ICG was compared with 1% TB and with control subjects. Additionally, no endothelial was found by Horiguchi et al in 10 patients who had 0.5% ICG intra-operatively compared with controls.[136]

Holley et al studied both human eye bank and rabbit corneas and found that an exposure to 0.5% ICG for up to 3 minutes caused no adverse effect to corneal endothelial function, ultrastructure, and viability.[134] On the other hand, Chang et al, in their rabbit corneal endothelial cells culture observed that a one-minute exposure to 0.5% ICG or higher resulted in significant increase in the percentages of damaged cells. They determined that a concentration of 0.25% ICG for 1 minute was safe.[38,40] The explanation may be that rabbit corneas typically are more susceptible to toxic insult than human corneas. The thicker glycoprotein-mucin layer coat-

TABLE 1B

Staining the Anterior Capsule: Experimental Animal Studies

Author et al (pub year)	Model Used	Level of Evidence	Dye Concentration (%)	Incubation Time (sec)	Injection Technique	Study Purpose	Intraocular Toxicity
Gamal Eldin (1999)[88]	Rabbits	III	Crystal violet 0.25; 0.5; 1.0; 2.0	NA	Under air bubble	Capsule stain and toxicity	NA
Unlu (2000)[377]	Rats and humans	III	GV 0.001; 0.01	NA	Under air bubble	Stain proprieties and toxicity	Not present
Holley (2002)[134]	In vitro	III	ICG 0.5	180	NA	Evaluate corneal toxicity	Not present
Van Dooren (2004)[380]	In vitro	III	TB 0.001; 0.005; 0.01; 0.1	NA	NA	Toxicity evaluation in corneal fibroblasts	TB 0.1 for 5 min presented corneal structural changes
Satofuka (2004)[323]	Porcine	III	TB 0.1 ICG 0.5	10	Under air bubble	Staining properties	NA
Wollensak (2004)	Porcine	III	TB 0.1	NA	Under air bubble	Biomechanical behavior of anterior capsule after staining	NA
Chang (2005)[38,40] Part 1 and 2	Rabbits	III	TB 0.001; 0.01; 0.1; 1 ICG 0.0025; 0.025; 0.25; 0.5 FS 0.63; 1.25; 2.50; 5 GV 0.001; 0.01; 0.1; 1 MB 0.001; 0.01; 0.1; 1	NA	NA	Investigate corneal toxicity and find the lowest effective concentration for CCC	Corneal endothelia toxicity present with ICG 0.5% GV 0.1% MB 0.5% or higher concentrations of the same dyes
Melendez (2005)[243]	In vitro	III	TB 0.0025; 0.004; 0.005; 0.0075; 0.008; 0.01; 0.025; 0.04; 0.2 ; 0.4 ICG 0.0025; 0.005; 0.0075; 0.01; 0.025; 0.05; 0.25; 0.5	NA	NA	Photodynamic actions on human lens epithelial cells	Toxicity to lens epithelial cells was found with ICG
Hisatomi (2006)[130]	Porcine and rats	III	BriB 0.001; 0.01; 0.025; 0.05; 0.1; 1.0	NA	Under air bubble	Anterior capsule staining ability and biocompatibility	Not present
Chang (2006)[39]	Rabbits	III	TA 0.4; 4.0	60; 180; 600; 1,800	NA	Toxicity evaluation in corneal endothelia	Present and related to TA vehicle

BriB = brilliant blue; CCC = continuous curvilinear capsulorrexis; FS = fluorescein sodium; GV = gentian violet; ICG = indocyanine green; IOL = intraocular lens; IOP = intraocular pressure; MB = methylene blue; NA = not applicable; PCC = posterior curvilineous capsulorrhexis ; TA = triamcinolone acetonide; TB = trypan blue.

ing human endothelial cells may provide added protection.

Photodynamic actions of ICG on human LEC were also evaluated in vitro. Doses above 0.1 mg/ml ICG were considered cytotoxic. After diode laser exposure, there is a dose-dependent photodynamic effect manifested as increasing loss of LEC viability. These results indicate that ICG might be a candidate for selective eradication of LEC to decrease the formation of posterior capsule opacification.[243]

Allen et al described a case of retinal toxicity after a complicated cataract surgery in which 0.5% ICG leaked to the vitreous cavity through zonular dehiscence. The patient developed a vitreous opacity during the surgery. On the fifth day postoperatively the patient presented with a visual acuity decreased to light perception pain. Pars plana vitrectomy was performed, and improvement of visual acuity 8 months after surgery.[4]

Fluorescein Sodium

Initial reports by Pandey and co-authors showed adverse intra-operative events with the use of FS in cataract surgery.[282,283] A posterior video/photographic technique demonstrated a leakage of FS into the vitreous both after FS was administered under an air bubble and by intracameral subcapsular injection. This was attributed to the low molecular weight of FS preventing the dye from being removed from the vitreous cavity by an irrigation/aspiration system. Although more serious complications have not been observed, FS may not be an optimal agent in aiding the CCC.

In regards to the safety of FS, corneal endothelial cytotoxicity with FS has been not demonstrated in in vitro investigations. FS in concentrations up to 10% have not resulted in loss of cellular integrity or disruption of organelles or cell lysis.[59] Further experimental and clinical experience will determine whether FS may be toxic to the corneal endothelial cells.

Gentian Violet

Unlu and co-authors concluded that GV can safely be used in human eyes, demonstrating no significant difference between the degree of corneal edema and IOP in concentrations of 0.01% and 0.001% during the 3 months of follow-up. However, concentrations of 0.1% or higher are toxic to cultured endothelial cells.[377]

Gamal Eldin et al found that 1% and 2% solutions caused severe, irreversible damage involving all corneal layers. The injury was less severe with 0.5% concentration; however, transmission electron microscopy revealed changes in the stromal keratocytes after one month.[88] Dada et al performed a clinical study comparing safety and efficacy of 0.1% TB, 0.5% ICG, 2% FS, patient's autologous blood, and GV 0.001% for anterior capsule staining in eyes with white cataract.[59] No significant difference regarding IOP, pachymetry, and endothelial cell loss were found among the dyes; however TB, ICG, and GV promoted greater visualization during CCC, providing quick and homogenous staining of the capsule. GV may be particularly suited for use in developing countries as it is cost-effective and easily available, but first its safety should be validated in a study with a larger cohort.

Brilliant Blue G

Hisatomi and co-authors tested brilliant blue G (BriB) biocompatibility in rat's eyes compared to TB and ICG.[129] In a light microscopic examination, no signs of endothelial cell loss or corneal edema were observed, even at 10 mg/ml. Lamellar collagen layers, stromal cells, and epithelial cell layer were well preserved. No inflammatory cell infiltration was observed in any corneal layers. Studies are needed to determine whether BriB is safe for human use.

Triamcinolone Acetonide

Cultured endothelial cells from rabbit's eyes were used to evaluate the toxicity of TA suspensions.[39] Benzyl alcohol, a vehicle of the commercial TA, and TA in the concentration of 40 mg/ml, with or without the vehicle, were shown to be toxic to corneal endothelium. The use of 4 mg/ml vehicle-removed TA for anterior vitreous staining is recommended to prevent corneal damage.

Methylene Blue

Brouzas et al reported a case of unintentional use of 1% MB for capsule staining during cataract surgery.[30] This resulted in severe corneal endothelial decompensation and iris pigment dispersion with development of bullous keratopathy and severe visual loss. Almost total destruction of corneal endothelium may have resulted from direct cytotoxicity or loosening of endothelial adherence to DM.

Report of the American Acadamy of Opthalmology

Jacobs et al recently published a report of the American Academy of Ophthalmology on capsule staining during cataract surgery.[153] The review included 36 articles separated according to the strength of evidence I to III (I for properly conducted randomized clinical trials) in which three important topics were approached. First, the effect of each dye

in staining the anterior capsule was evaluated and compared with each other. They found level III evidence that ICG, TB, and FS were effective in staining the capsule, whereas ICG and TB provided better ease of use and visualization of the capsule than FS. A second topic reviewed was the role of vital dyes as an adjuvant in cataract surgery; the reviewers found stronger level II evidence in pediatric cases of patients less than 5 years of age and in cases of white cataracts. The third topic investigated the safeness of the dyes as a staining agent; TB was considered safe, ICG and FS need more long term studies and crystal violet and GV considered toxic for capsule staining. They concluded that appropriate vital dyes are safe and effective as an adjunct for capsule visualization in cataract surgery, especially in cases with inadequate red reflex.

The Use of Vital Dyes in Vitreoretinal Surgery—Chromovitrectomy

RATIONALE

Internal limiting membrane (ILM) peeling to treat idiopathic macular holes (IMH) was first described by Eckardt et al in 1997.[68] With the use of this ground-breaking technique, closure rates in MH surgeries of approximately 95% have been reported,[3,28,29,95,248,277,284,365] compared with 58–94% closure rates in eyes without ILM-peeling.[97,173,234,315,367,394] A large meta-analysis of published nonrandomized studies involving 1,654 eyes suggested that ILM-maculorhexis increases anatomic (from 77% to 96%; $p < 0.0001$) and functional (55–81%, $p < 0.0001$) success rates of IMH surgery.[248] However, surgical removal of ILM may lead to retinal damage.[361,374] The two main complications of ILM removal are visual field defects and RPE damage.[9,165,242,251,273,392,393] Ito et al reported damaged to the nerve fiber layer in 46% of patients submitting to ILM peeling that did not present corresponding alterations in microperimetry.[145,146]

Visual field defects after ILM removal are thought to be caused by surgical trauma. Visual field defects mainly located temporal to the macula could be related to mechanical trauma to optic disc, to the fluid–air exchange or to direct trauma to the retina.[242,273] Welch et al postulated that retinal dehydration after air–fluid exchange produces visual field defects.[392,393] Other hypotheses are that the maneuvers that produce surgical poseterior lead to visual field defects[52] or that high intraocular pressure during fluid–air exchange causes optic disc damage.[242]

Macular retinal pigmentary epithelial hyperpigmentation or hypofluorescence on angiography could be induced by surgical trauma or phototoxicity.[161,255] In one study involving 15 patients, RPE abnormalities after pars plana vitrectomy were documented in all cases. This has been interpreted as iatrogenic punctate chorioretinopathy in the area touched by the forceps.[166]

The use of vital dyes to stain pre-retinal tissues during vitreoretinal surgery, "chromovitrectomy", allows visualization of the thin, transparent tissues in the vitreoretinal interface: the ILM, epiretinal membrane (ERM), or the vitreous posterior surface.[285] Abrams et al reported the first use of vital dye during vitreoretinal surgery and found fluorescein a great aid in vitreous identification.[1] This technique was largely ignored for several decades; however, since 2000 chromovitrectomy has achieved widespread use.[32] Initially, intravitreal injection of ICG facilitated the visualization of the ILM.[306–310] Later, TB was proposed as a helpful tool to identify the several types of ERM, and TA was found to stain the vitreous.[221] Recently, other dyes, including IfCG, PB, BroB, and BriB, have been proposed as alternatives for chromovitrectomy.[306,308,309]

VITAL DYES FOR INTERNAL LIMITING MEMBRANE STAINING

Indocyanine Green

ICG adheres well to the extracellular matrix components of the ILM, such as collagen type 4, laminin, and fibronectin.[62,63,93] Wollensak et al showed, in a porcine model, that ICG with light exposure produces a significant increase in biomechanical stiffness, thereby facilitating ILM peeling.[402] Following the Kadonosono et al publication of ICG use macular hole surgery, many authors have reported easier and less traumatic ICG-guided peeling with good clinical results (Fig. 3).[54,89,91–93,158,161,195,197,202,204,205,207,222,353,391] Clinical data showed that macular closure rate may be achieved in 74–100% of patients using ICG-guided ILM peeling.[8,20,54,55,75,115,138,141,158,193–195,197,210,211,217,224,239,254,255,271,274,298,305,306,309,310,313,325,326,329,337,345,370,371,375,379,389,405]

The potential for toxic effects of ICG on the retina has been suggested.[73,96,114,115,228–230] ICG may persist after macular hole surgery for up to 36 months.[11,48,174,255,325,326,334,351,390] In addition, ICG could also migrate to subretinal space through the MH, causing retinal damage.[9,28,79,128,351,359,390] Complications of ICG-assisted chromovitrectomy include RPE changes,[73,128,165,298,327,371] visual field defects,[164,370,416] and optic nerve atrophy.[128,228–230,307,308,371]

Few controlled studies have been performed to compare ILM removal with and without ICG staining in MH surgery. Some authors found significantly

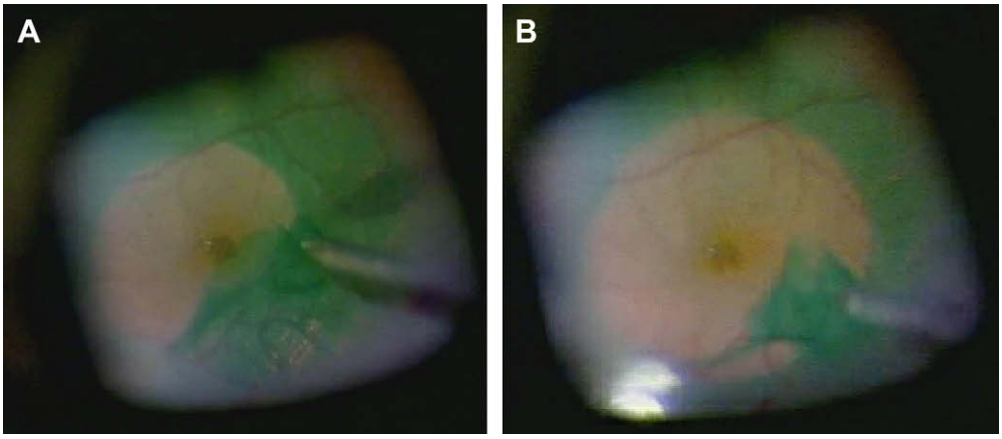

Fig. 3. ILM-staining with ICG in macular hole surgery. *A:* The green-stained ILM is easier to view and peel, since the green cyanine dye changes the biomechanics of the fine ILM. *B:* Continuous removal of the ILM enables assurance that all antero-posterior and tangenctial traction is removed, although some dye may remain in the eye when the dye is injected onto the posterior pole.

worse visual outcomes when ICG was used as an adjuvant to ILM-peeling in the treatment of idiopathic MH.[26,27,138,337] A recent randomized, prospective study comparing ILM peeling with and without ICG staining for idiopathic ERM,[126] however, found no difference in preoperative or postoperative visual acuity, reduction of macular edema, or incidence of recurrent ERM. The better outcomes in recent papers may be explained by a more careful ICG-injection technique.[194,224,239,313,345]

In comparing studies of ICG-assisted MH surgery criteria such as dye incubation time, concentration and osmolarity should be noted. Incubation time, the time that the dye remains in the vitreous cavity before aspiration, may vary from immediate removal to 5 minutes. RPE changes have been found mainly in studies reporting ICG incubation time over 30 seconds. RPE toxicity is more common when the ICG solution has an osmolarity below 270 mOsm and concentration above 0.5% (Table 2).[7,8,20,54,55,73,110,114,115,138,141,158,193–195,202,204,205,207,210,217,224,236,239,255,298,305,329,337,353,359,371,389,391]

The first use of ICG in MH surgery was with a dye concentration of 0.5%.[55] After RPE changes and visual field defects appeared, a lower concentration was thought to be safer.[7,306,307] Lai et al, in their retrospective study of 49 eyes using low ICG concentration (0.125%), found no signs of retinal toxicity, with good anatomical and visual results.[211] In contrast, Engelbrecht et al, using the same ICG concentration, observed 54.5% with RPE changes.[73] The difference could be related to the solution osmolarity, which was 299 mOsm in the former and 250 mOsm in the latter.[73,211] Maia et al observed 27.5% of RPE abnormalities after 0.5% of ICG used with osmolarity of 270 mOsm. This high rate of RPE abnormalities may be related to the ICG concentration, technique of application, ICG exposure to light, or a combination of factors.[228] Recent studies have utilized ICG in a concentration of 0.05% and osmolarity around 290 mOsm with few or no signs of RPE toxicity.[136,146,217,305]

We did a meta-analysis of ICG application for ILM-peeling in 837 eyes that showed similar anatomic, but worse functional, outcomes when ICG has been used in chromovitrectomy.[310] Most of the studies in this meta-analysis used ICG in high concentrations and volumes.

ICG has been also used to facilitate ILM peeling in other diseases. Kamura et al evaluated the use of ICG-assisted ILM peeling in diabetic macular edema (DME). No sign of retinal toxicity were seen, and visual outcome was similar to vitrectomy without ICG.[163] Recently, Bardak et al compared ICG to TA in patients with diffuse DME. No difference was shown between the two groups.[17] Radetzky et al evaluated ILM peeling with ICG for persistent macular edema from causes such as central retinal vein occlusion, DME, Irvine-Gass syndrome, and vitreomacular traction syndrome. Significant improvement in visual acuity was observed only in patients with DME.[299] A large-scale randomized trial should evaluate the benefit and safety of ICG-guided ILM peeling in other diseases.[111,161,163,189,274,299]

Infracyanine Green

IfCG also binds with high affinity to the acellular ILM. Ullern et al demonstrated that IfCG stains ILM homogenously,but not epiretinal membranes (a cellular tissue).[143,144,182,186,270,276,376,391] The iodine-free IfCG should be dissolved in 5% glucose solvent generating an iso-osmotic solution of 294–314 mmol/kg. Indeed, osmolarity changes at the

TABLE 2

Different Surgical Techniques to staIn ILM with ICG in Macular Hole Surgery

Author et al (pub year)	Evidence Level	Number of Eyes	Type of Macular Holes	ICG Concentration (%)	Osmolarity of the ICG Solution (mOsm)	ICG Dissolved In	Volume of ICG Admnistered IV (ml)	Concentration of ICG IV (%)[h]	ICG Incubation Time (sec)	Anatomic success[a]	Visual Acuity Improvement (%)[b]	Complications Related to the Dye/ILM Peeling
Kadonosono (2000)[158]	III	13	Idiopathic	0.06	270	Water/ Viscoelastic	ND	[c]	30	92	84	No
da Mata (2001)[55]	III	24	Idiopathic/ Traumatic	0.5	270	Water/ BSS	0.2–0.4	0.025–0.050	180–300	88	83.3	No
Kwok (2001)[207]	III	10	Idiopathic	0.025	293	Water/ BSS	ND	[c]	30	100	70	Retinal elements adherent to the ILM on histology
Stalmans (2001)[353]	III	4	Idiopathic	0.33	ND	Glucose 5%	0.1–0.3	0.008–0.0247	180	ND	ND	Glial cell-like processes adherent to the ILM
Engelbrecht (2002)[73]	III	21	Idiopathic	0.1	250	Water/ BSS	1–2	0.025–0.050	30–150	86	19	54.5% RPE changes
Schmidt (2004)[329]	III	32	Idiopathic	0.5	270	Water/ BSS	0.1–0.2	0.0125–0.025	5	ND	ND	No
Kumar (2002)[195]	III	18	Idiopathic	0.5	ND	Water	0.1	0.0125	120	83.3	61.1	No
Weinberger (2002)[391]	III	18	Idiopathic	1:9 in sodium chloride 0.9%	ND	Sodium chloride 0–9%[g]	0.15–0.30[j]	0.001–0.002[j]	60	78	78[d]	Strong signal with SLO in all cases
Haritoglou (2003)[111]	III	20	Idiopathic	0.05–0.50	275	Water/ BSS	0.2–0.5	0.0025–0.0625	60	90	45	35% nasal visual field defects; cellular elements adherent to the ILM
Kwok (2003)[202]	III	41	Idiopathic	0.025; 0.050; 0.125	292, 295, 299	BSS	0.2	0.0012–0.0062	30	87.8	58.5[e]	No
Van de Moere (2003)[379]	III	51	Idiopathic/ Traumatic	infracyanine 0.5	309	Glucose 5%	0.2	0.025	120	92	74.5	No
Sheidow (2003)[337]	III	35	Idiopathic	0.41	ND	Water/ BSS	ICG wash out	[c]	30	97.1	71.4	No
Tadayoni (2003)[359]	III	17	Idiopathic	0.25	ND	Glucose 5%/ BSS	0.1–0.2	0.0062–0.0125	180	ND	94.1[d]	Persistence of fundus fluorescence with infrared examination
Sato (2003)[332]	III	23	Idiopathic	0.06	270	ND	ND	[c]	30	91.3	82.6[e,f]	No
Kwok (2003)[205]	III	18	Idiopathic	0.025; 0.050; 0.125	292, 295, 299	BSS	ND	[c]	30	88.9	66.7	ERM development, no RPE changes
Kromer (2004)[193]	III	20	Idiopathic	0.5	ND	BSS	0.3	0.375	180	87%	ND	No

Ando (2004)[7]	III	16	Idiopathic	0.05	ND	BSS	0.1–0.2	0.0125–0.025	10	93.7	ND	No
Ben Simon (2004)[20]	III	16	Idiopathic	0.25	ND	ND	0.1	0.0625	20	81	85	No
Da Mata (2004)[54]	III	16	Idiopathic	0.5	270	BSS	0.05–0.1	0.0625–0.125	30	98	96	12.5% of RPE changes
Horio (2004)[138]	II	40	Idiopathic	0.125	270	BSS	0.2	0.005–0.006	10–30	100	100	No
Lai (2005)[210]	III	20	Idiopathic	0.1	299	Glucose 5%	0.1	0.025	60	95	85	No[i]
Lee (2005)[217]	II	19	Idiopathic	0.5; 0.05	242; 295	BSS	0.5	0.0625–0.625	30–60	85.9	ND	ERM development
Nakamura (2005)[255]	III	18	Idiopathic	0.5	270	BSS	ICG wash out	[c]	Immediate removal	100	ND	RPE changes in 5.5%[i]
Posselt (2005)[298]	III	14	Idiopathic	0.5	270	BSS	0.2–0.4	0.25–0.5	60–180	92.8	35.7	50% RPE changes
Wang (2005)[389]	III	17	Idiopathic	0.5	ND	BSS	0.2	0.25	30	88.2	17.6	No
Ferencz (2006)[75]	III	21	Idiopathic	0.125	270	BSS	0.2	0.005–0.006	Immediate removal	100	100[d]	No
Kumagai (2006)[194]	II	96	Idiopathic	0.1	ND	BSS	0.1–0.2	0.025–0.05	Immediate removal	99	81	No
Mavrofrides (2006)[239]	II	83	Idiopathic	0.25	ND	BSS	0.2–0.3	0.125–0.185	10	87	59	No
Rizzo (2006)[305]	III	31	Idiopathic	0.05	309	Glucose 5%	ND	ND	10	97	ND	No[i]
Husson-Danan (2006)[141]	III	23	Idiopathic	0.08–0.05 infracyanine green	295	Glucose 5%	0.05	0.00625–0.01	30	74	ND	RPE changes in 10%, visual field defect in 7%
Lai (2007)[211]	III	59	Idiopathic	0.125	ND	BSS	0.05	0.0156	20	98	73	No
Nagai (2007)[254]	III	35	Idiopathic	0.5	ND	BSS	0.05	0.0625	Immediate removal	97	56	Foveal RPE atrophy (0.7%) and visual field defects (2.4%)
Tsuiki (2007)[370]	III	96	Idiopathic	0.25	ND	ND	0.1–0.2	0.0625–0.125	Immediate removal	ND	ND	Visual field defects (17.7%)
Rufer (2007)[313]	III	36	Idiopathic	0.05	ND	Glucose 5%	0.2–0.3	0.025–0.0375	Immediate removal	80.5	ND	No
Ullern (2007)[375]	III	21	Idiopathic	Infracyanine 0.25	306	Glucose 5%	1.0–2.0	0.625–1.25	60–120	95	86	RPE changes in 14.2%

BSS = Balanced salt solution; I.V. = Intravitreal; ND = no data.

[a] With one surgery and defined as disappearance of the fluid cuff.

[b] Defined as two or more Snellen lines visual acuity improvement after one surgery.

[c] Could not be calculated.

[d] One or more line visual acuity improvement.

[e] Several surgeries.

[f] Visual acuity improvement of 20/50 or better.

[g] A membrane filter was used to exclude precipitation.

[h] Assuming that the vitreous cavity has 4 ml.

[i] Used autologous whole blood to protect macular hole.

[j] Assuming that one drop contains 0.075 ml.

vitreoretinal interface or subretinal space induced by various types of solutions have been shown to be toxic to retinal cells and tissue.[132,235,263,355]

Several recent clinical investigations have shown positive results with IfCG application with little or no retinal toxicity.[112,186,209,213,218,304] IfCG-assisted ILM peeling demonstrated a high closure rates of macular holes (over 90%) and an improvement in visual acuity.[112,151,186,209,213,218,304] In diabetic eyes undergoing surgical ILM removal for macular edema, IfCG appeared to be safe and to promote good clinical results.[189] Controlled clinical studies showed that IfCG-guided peeling did not significantly improve the results of macular hole surgery.[140,141]

Immunohistochemical analysis of the ILM peeled with IfCG show GFAP and S-100 positive staining, indicating the presence of remnants of footplates from Muller or glial cells and neural or ganglion cells, respectively.[209] This could explain why, in cadaver eyes, IfCG as well as ICG followed by illumination may alter the cleavage plane at inner retinal layers of the ILM.[108,112,113] Therefore, although IfCG and ICG may facilitate ILM removal, they may produce unwanted retinal alterations in the neurosensorial RPE and lead to visual field defects.[108,112,113,165,174,239,392,393] In summary, a safer IfCG profile may make it a better alternative than ICG for chromovitrectomy in humans, since IfCG in the concentration of 0.5 mg/ml allows ILM identification with fewer toxic effects.

Brilliant Blue

In humans, BriB produced appropriate ILM staining in an iso-osmolar solution of 0.25 mg/ml when used for idiopathic ERM and MH treatment. A total of 85% of eyes improved at least two Snellen lines, with no signs of toxicity.[70,71] Cervera et al reported similar outcomes, with good ILM staining and clinical results and no signs of toxicity on multifocal electroretinogram (ERG).[36] BriB is emerging as a good alternative for ICG and IfCG in chromovitrectomy because of its remarkable affinity for ILM, although the data on its toxicity are limited.

Bromophenol Blue

BroB is proposed as an alternative biostain for chromovitrectomy. Comparison of six biological stains (light green yellowish, E68, Chicago blue, rhodamine, rhodulinblau-basic, and rhodulinblau-basic 3) revealed that BroB stained the ERM and ILM better, and induced neither in vitro damage on ARPE-19 nor primary RPE-cell proliferation at concentrations of 0.2% and 0.02%.[117,120] Further in vivo studies in rodent and porcine eyes demonstrated that BroB at concentrations of 0.5% and 0.02% promoted less retinal toxicity as assessed by histology and ganglion cell counts in comparison to three other vital dyes (Light-green, Chicago blue, and E68). Moreover, BroB at concentrations of 1% and 2% promoted enhanced ILM coloring and identification.[119,120,330] Further human clinical data should elucidate the best indication of BroB in chromovitrectomy and its safety in comparison with other current dyes available.

Triamcinolone Acetonide

Kimura et al first used TA for ILM peeling, observing that the white specks and crystals deposit over the ILM, thereby facilitating its identification and removal.[181] They obtained good clinical results and observed no adverse effects after three months. Subsequent pathology disclosed the presence of ILM in specimens after TA-assisted peeling, thereby showing that is possible to remove ILM and not just vitreous.[318,335,336,360] Since then other studies have confirmed that TA-assisted ILM removal provides good results.[137,162,335] Compared to ICG-guided ILM removal, a non-controlled study indicates that TA has the same closure rate with significantly better visual results with no side effects.[166]

Crystals of TA have been detected up until 40 days post surgery with chromovitrectomy for MH surgery.[84] Some authors suggest that this persistent TA could slow the healing process necessary for MH closure.[84,191,411,412] Recently, Kampougeris et al compared 18 cases of ILM peeling with and without the use of TA. During a mean follow-up of 7.3 months, no recurrence of the MH was observed.[162] Despite this concern, preservative-free TA appears to be a preferred agent in chemovitrectomy.[227]

Trypan Blue

Soon after its introduction in cataract surgery, TB was proposed as a stain for chromovitrectomy.[382] However, TB does not enhance ILM-visualization as well as ICG; and TB is recommended mainly for ERM-staining.[306,309] Our experience confirms that ILM visualization with TB is much more difficult than with ICG-guided.[217,221,306,308,309] To enhance dye penetration onto retinal surface in an air–fluid exchange, TB may be mixed with glucose, thereby creating a dye solution denser than water. Lesnik Oberstein et al used TB and 10% glucose isovolumetrically in osmolarity of 320 mOsm/l to evaluate ERM staining without fluid–air exchange.[218] In 29 eyes, just 25% needed a reapplication of the solution to achieve ERM staining. In addition, all patients exhibited improved visual acuity with no

signs of retinal toxicity. However, higher glucose concentrations should be avoided.[235,263,264] Marmor reported in animals that injection of 0.05 ml of a 1,000 mOsm solution caused rapid whitening of the posterior retina, followed by the development of a large detachment and permanent retinal degeneration.[235] An ERG showed immediate loss of the c-wave and a slower decline of the a- and b-waves. Osmolarity should be considered in planning any vitreous injection of dyes and drugs.

Many clinical studies reveal that TB exerted little or no toxic effect to the retina.[289,290,354–356,363,385,386] Two comparative studies evaluated the anatomical and visual outcomes after vitrectomy and ILM peeling for treatment of patients with stage II to IV idiopathic MH using ICG or TB. Their rate of MH closures was the same; however, vision was significantly better only in the TB group.[21,217] Histological examination discloses that TB staining may produce retinal damage, especially at higher concentrations.[87,108,155,201,208,258,259,303] In the future, clinical investigations should clarify the role of TB in combination with other vital dyes in chromovitrectomy, the so-called double staining, and elucidate the safe dose of intravitreal TB for chromovitrectomy.[356,410]

VITAL DYES FOR EPIRETINAL MEMBRANES STAINING

Trypan Blue

TB exhibits a strong affinity for ERM because of the many dead glial cells within those membranes.[13–15,306,309] Various investigators, including our group, agree that TB is indicated for recognition of ERMs in vitrectomy, as the blue dye enables complete identification of the entire ERM surface (Table 3).[76,221,261,289,290,306] TB staining of the ERM may minimize mechanical trauma to the retina during ERM-removal and allows recognition of the extent of the ERM (Fig. 4).

Haritoglou et al investigated functional outcomes of macular pucker surgery with and without the use of 0.15% TB for a mean follow-up of 4–6 months in 20 patients. Postoperatively median visual acuity difference between the two groups was not statistically significant; however, 4 of 10 patients without and 7 of 10 patients with TB-staining experienced an improvement of visual acuity of two lines or more.[108,112,113] One comparative study with TB and ICG for ERM removal also gave results favoring the application of TB.[201,208]

No RPE defects or signs of retinal toxicity have been reported in most studies.[108,112,113,221] However, in one case report of a complicated ERM surgery, subretinal migration of TB away from the fovea accidentally occurred. Some RPE changes were noted at the migration site, but did not compromise visual acuity.[378] Histopathological analysis of excised ERM showed no retinal cells on the retinal side of the ERM and no signs of apoptosis.[13,14] Multifocal ERG found no retinal toxicity.[15] Finally, transmission electron microscopy of the TB-stained ERM showed fragments of ILM in all specimens.[201,208] Interestingly, Smiddy and coworkers showed that without the use of any dye, epiretinal membrane fragments contain ILM fragments in 76% of cases.[346] The clinical relevance of those ultrastructural findings remains to be determined; however, future controlled studies should clarify if TB has any toxicity.

Patent Blue

Animal studies and preliminary clinical data demonstrate moderated affinity of PB to ERM and vitreous, but poor affinity to the ILM.[306,309] Nevertheless, our recent clinical data revealed that PB is as appropriate a vital dye for coloring ERM as TB.[246,247]

There is conflicting data regarding the retinal toxicity of PB.[266] In one study PB induced only mild and reversible retinal toxicity,[227] whereas RPE cells exposed in vitro to PB showed no toxicity.[246,247] Our rabbit model demonstrated no RPE defects on FA related to the subretinal PB injection and was similar to the control-BSS group.[232] Histologically, subretinal injection of PB resulted only in mild ultrastructural retinal damage during follow-up. The histological damage induced by TB was more severe than by PB. Although no definite conclusion can be drawn, most studies to date indicate a safer profile for PB compared to TB, particularly in neuroretinal cells. The safe dosage range for intravitreal PB injection, however, remains unclear.

Indocyanine Green

ICG has been proposed to allow better visualization of ERMs in vitrectomy for proliferative diabetic vitreoretinopathy (PDVR), idiopathic ERMs, and proliferative vitreoretinopathy (PVR).[126,188,200,307,308] The green dye may stain the acellular ILM best, however, and ERM-staining by other vital stains may be better.[306] Foster et al described a case of recurrent macular hole surgery where ICG stained ILM except in an inferior area with an ERM.[80] The same phenomena was observed in ERM surgery for PVR, where ICG stains ILM but not the ERM facilitating the identification and removal by negative staining.[319]

A retrospective study comparing ERM peeling with and without ICG showed no difference in visual

TABLE 3

Different Surgical Techniques to Stain Pre-retinal Tissues with TB in Vitreoretinal Surgery

Author et al (pub year)	Evidence Level	Number of Eyes	Ocular Disease	TB Concentration (%)	Osmolarity of the TB Solution (mOsm)	TB Dissolved In	Volume of TB Admnistered IV (ml)	Concentration of TB IV (%)[b]	TB Incubation Time (seconds)	Infusion Method	Visual Acuity Improvement (%)[a]	Complications Related to the Dye
Feron (2002)[76]	III	10	PVR	0.06	309[c]	None	0.5	0.075	60	Fluid–air exchange	ND	No
Perrier (2003)[289]	III	18	MH	0.06	309[c]	None	0.5 – 1	0.075 – 0.15	ND	Continuous infusion under BSS	56	No
Perrier (2003)[290]	III	23	ERM	0.06	309[c]	None	0.5 – 1	0.075 – 0.15	ND	Continuous infusion under BSS	74	No
Li (2003)[221]	III	14	MH / ERM	0.06	309[c]	None	0.5	0.075	120	Fluid–air exchange	57	No
Teba (2003)[363]	III	50	MH / ERM / PVR	0.2	329	None	0.1	0.05	60	Fluid–air exchange	ND	No
Haritoglou (2004)[108]	II	22	MER	0.06	309[c]	BSS	0.1	0.075	60	Fluid–air exchange	72.7	No
Haritoglou (2004)[112]	III	10	MER	0.15	293[d]	None	0.5	0.02	60	Continuous infusion under BSS	70	No
Vote (2004)[386]	III	26	MER / MH / PVR	0.15	293[d]	None	0.1	0.037	60	Fluid–air exchange	ND	No
Lee (2005)[217]	III	16	MH	0.15	293[d]	None	ND	ND	60	Continuous infusion under BSS	61.1	No
Balayre (2005)[15]	III	14	ERM	0.15	293[d]	None	0.2	0.075	120	Fluid–air exchange	100	No
Beutel (2007)[21]	II	20	MH	0.15	293[d]	None	0.1	0.037	Imediately removal	Continuous infusion under BSS	63	No

BSS = Balanced salt solution; ERM = epiretinal membrane; I.V. = Intravitreal; MH = macular hole; ND = no data; PBS = phosphate buffer sodium; PVR = proliferative vitreoretinopathy.

[a]Defined as two or more Snellen lines visual acuity improvement after one surgery.

[b]Assuming that the vitreous cavity has 4 ml.

[c]Information of in stock solution of VisionBlue (DORC, Zuidland, The Netherlands).

[d]Information of in stock solution of MembraneBlue (DORC, Zuidland, The Netherlands).

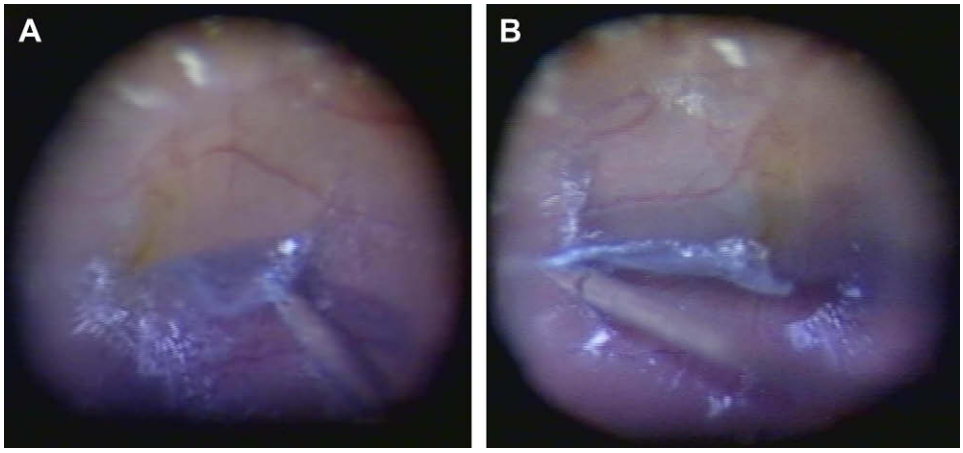

Fig. 4. Staining the epiretinal membranes (ERM) with trypan blue (TB) after TA-guided vitreous removal. *A:* The blue dye TB allows creating an edge between the stained ERM and the unstained retinal surface. *B:* The retina is easily cleared of ERM and traction, amd TB-guided ERM staining also enables assurance that some remaining ERM still persists.

acuity between the groups.[188] A controlled clinical study evaluated patients that underwent ERM surgery with or without ICG. No difference in visual acuity, recurrence of ERM, or retinal toxicity was observed.[125,126] More recently, a randomized prospective study investigated the effect of ILM peeling with and without ICG staining for idiopathic ERM removal. There was no difference in preoperative or postoperative visual acuity, reduction of macular edema, or incidence of recurrent ERM between the two groups.[125,126]

SURGICAL TECHNIQUES FOR DYE APPLICATION DURING VITREORETINAL SURGERY

Dye Injection

Several different techniques have been used to inject vital dyes in the vitreous cavity.[53] One has been named the "dry method" or "air-filled technique". By either name, this consists of removing the fluid in the vitreous cavity by a fluid–gas exchange before dye injection. Although this 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.[307,308] When the eye is air-filled, the full concentration of dye injected into the vitreous cavity reaches the retinal surface. The second technique is called 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 concentration of the dye in contact with the retinal surface is lower because it is diluted by the fluid in the vitreous cavity. The disadvantage to this technique is the possible dispersion of the dye leading to unwanted staining of the retina elsewhere. Czajka and colleagues compared the two methods in a porcine model and concluded that the air-filled technique induced a higher incidence of RPE atrophy and outer retinal degeneration.[53]

A further concern is incubation time of the dye on the retinal surface. Because early dye washout minimizes exposure on retinal tissue, there is a trend to wash out the dye a few seconds after its injection.[329]

To avoid an unnecessary and non-selective staining of the entire retina, we developed a new applicator for chromovitrectomy. A prototype was constructed of a metal cannula enclosed in an adjustable silicone tube, giving the instrument a 20-gauge outer diameter, with a brush-like proximal end containing multiple long silk filaments. Subsequently, a commercially available instrument called VINCE (Vitreoretinal INternal limiting membrane Color Enhancer; Dutch Ophthalmic, Zuidland, The Netherlands) has been produced. It consists of a modified backflush needle containing an adjustable silicone tube, which is surrounded by metal cannula. This new device may allow a better visualization of fine, delicate, semitransparent pre-retinal tissues, while avoiding the uncontrolled staining of the RPE in the MH and peripheral retina.[249]

Macular Hole Protection

There are some ways to avoid dye injection directly through the MH: slow injection of the dye, selective painting instrument (VINCE),[249] or placing substances over the MH such as perfluorocarbons liquids (PFCL),[74,278,332] autologous whole blood,[210,305] or sodium hyaluronate,[34,61,197,215,294,317] In chromovitrectomy, PFCL has been used as a protective agent for the macular hole to avoid the entrance of ICG into the subretinal space.[74,278,332] Olson et al described

this surgical approach and proved the immiscibility of PFCL and ICG in vitro.[278] Scupola et al reported the one-year outcomes of eight patients using chromovitrectomy with PFCL and found no signs of RPE defects and a final median best-corrected visual acuity of 20/50.[332] The use of PFCL increases the costs and operative time, and the meticulous use of a small-tip fluted needle is essential to prevent retained PFCL that could lead to retinal toxicity.[278]

Autologous blood has been used as a protective agent during ICG-assisted macular hole surgery.[210,305] Lai et al suggested that, at least in vitro, blood could partially protect RPE cells from ICG toxicity.[210] The authors observed that reduced cell viability was greater in the group without blood exposure and no difference was observed when comparing whole blood, plasma, or packed red blood cells. Clinically, small uncontrolled case series showed that whole blood used in ICG-assisted vitrectomy for macular hole surgery is apparently safe, with no signs of retinal damage or residual ICG after one month.[305]

Kadonosono et al[158] first described the use of viscoelatics for this purpose. Viscoelastic can be used to control where the dye settles on the retinal surface to avoid staining outside the macular region.[34,197,317] Although good visual and anatomic outcomes could be achieved with this technique, it requires more surgical time.

SUBRETINAL VITAL DYE FOR FACILITATION OF RETINAL BREAKS VISUALIZATION

Rationale

Exact localization of retinal breaks is a critical step in the surgical treatment of rhegmatogenous RD.[150] Despite clear visualization of the fundus, in 2.2–4% of phakic RDs, the retinal breaks may not be found. In aphakic and pseudophakic RDs, the incidence of nonvisualized breaks may be even higher, ranging from 7% to 16% and 5% to 22.5%, respectively.[321]

Early in the 20th century, numerous experiments were performed to investigate staining retinal breaks during retinal surgery.[223,306] In 1939, Sorsby used intravenous Kiton-Fast-V-Green in patients with RD to detect retinal tears. He observed a greenish retina with an unstained retinal break.[350] In the 1950s other researchers described the use of intravenous fluorescein for the same procedure and described a colored edge in the tears.[270,343]

Clinical application of vital dyes for subretinal breaks identification

The first use of subretinal application of dyes to stain retinal breaks was made in 1947 by Black. He used methylene blue through a transcleral needle.[25] This vital dye application was considered unsuccessful by Hruby and Gass because of its absorption in the retinal pigment epithelium.[140] Later, Kutschera showed that systemic infusion of PB enabled both understanding of retinal metabolism and absorption in animals, which provided further evidence for the role of staining agents in the recognition of retinal breaks.[198] Recently, Jackson applied subretinal 0.15% TB with a 41-gauge cannula to identify retinal breaks in patients with RD and no identifiable tears during surgery. Retinal breaks were identified in four of the five patients, and no retinal toxicity was seen in this study. However, the small number of patients does not allow assessment of the risks and potential toxicity.[148,149]

VITAL DYES FOR VITREOUS STAINING DURING VITREORETINAL SURGERY

The vitreous plays a very active and important role in several vitreoretinal diseases, including macular holes, macular edema, and diabetic retinopathy. In vitreoretinal surgery for therapy of those diseases, complete removal of the vitreous gel may enhance surgical outcomes.[333]

Triancinolone Acetonide

TA deposition onto the vitreous surface was initially reported by Peyman et al.[293] The crystals of the crystalline steroid adhere to the acellular tissue, thereby enabling a clear contrast between the empty vitreous cavity and the areas where the vitreous fibers are still present.[33,106,194,295,318,320] The currently technique for TA application consists of a simple injection of the agent into the vitreous cavity. Following the initial report, a number of studies confirmed the efficacy of TA for staining the transparent vitreous, whereas anatomic or functional signs of complications have rarely been observed (Fig. 5).[17,44,66,84,85,137,154,162,166,181,191,318,320,335,336,349,360,411,412] In addition to its effect on the vitreous visualization, a TA injection during vitrectomy may prevent fibrin reaction and post-operative PVR. A recent multicenter, controlled clinical trial performed in Japan investigated the use of intraoperative TA during vitreoretinal surgery and found decreased risk of post-operative RD, but increased need for post-operative anti-glaucoma eye drops, with TA application.[409]

Sodium Fluorescein

Hydrophilic sodium fluorescein (SF) is exceedingly well absorbed by the vitreous. Das and Vedantham showed that intravitreal SF 0.6%, compared to 20% injectable dyes, improved the visualization of clear vitreous fibers during chromovitrectomy, with no complications.[60] Guo et al compared four

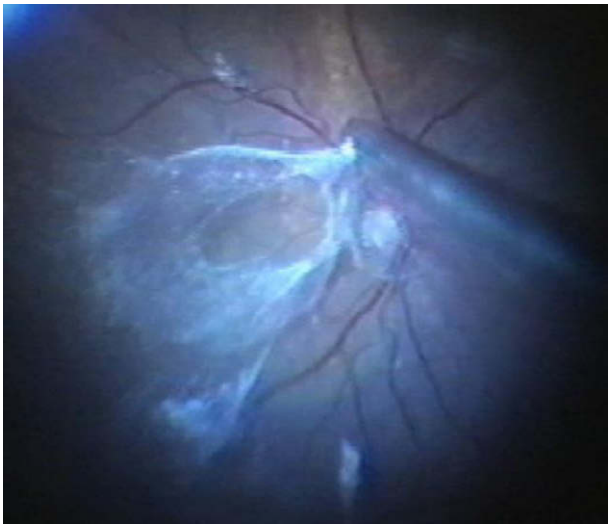

Fig. 5. Triamcinolone acetonide deposits to the acellular vitreous gel, providing a clear visualization of the posterior vitreous cortex.

different vital vitreous stains and found that fluorescein was inferior to the TA.[106] Currently, the main use of SF in chromovitrectomy is vitreous staining. Future clinical investigations should determine its role to improve visualization of membranes during vitreoretinal surgery.

Fluorometholone Acetate

The white steroid may be indicated for use in the treatment of steroid-responsive inflammatory conditions of the conjunctiva, cornea, and anterior segment of the eye. The safety of intravitreal or subretinal FMA to retina has been recently examined in rat and primate eyes. The researchers found neither remarkable reduction in any ERG waves, nor histological changes, and concluded the steroid FMA was a useful alternative to TA during chromovitrectomy.[122] Further clinical experience should elucidate the advantages and disadvantages of FMA in comparison to TA and other newer vital dyes.

Trypan Blue

Intracameral or intravitreal injection of TB has been used to highlight vitreous.[106,385,386] The blue dye in various doses may improve detection of both prolapsed vitreous in the anterior chamber and that remaining in the vitreous cavity. In one retrospective clinical series, 0.15% TB used for staining the vitreous produced no toxicity.[386] Verma et al claimed that TB may allow visualization of the margins of the vitreous strands during vitreoretinal surgery.[385] However, in one comparative analysis TB stained the vitreous less well than TA and FS.[106] For this reason TB application for vitreous visualization has not gained much popularity.

EXPERIMENTAL INVESTIGATIONS FOR EVALUATION OF DYE-INDUCED RETINAL TOXICITY

Clinical assessment—visual acuity, fundus examination, ERG, and fluorescein angiogram—have commonly been used to determine adverse retinal effects of intravitreally administered chemical substances and drugs.[43,64,238,295] Although several studies have demonstrated that good visual function can be attained after ICG-assisted vitrectomy,[54,391] other investigators report signs of toxicity such as visual field defects, RPE changes, and loss of lines in ETDRS-chart.[100,114,118,119,380,381] Histopathology of ILM-tissue during chromovitrectomy with ICG revealed either "no" or "some" cellular structures over and under the removed ILM.[209,256] The presence of retinal elements such as remnants of Müller cells, myofibrocytes, and astrocytes adherent to retinal surface of ILM after ICG-staining raised concerns of possible retinal damage due to a deeper cleavage plane in ILM-dissection.[90,92]

Experimental evaluation of retinal toxicity in animals

Electrophysiological testing is an effective and objective method for assessing the status of the visual pathways.[237] Currently, the basis of retinal evaluation for pharmacological and toxicological effects of intravitreally administered drugs and dyes in animals consists of the ERG and histopathology by light microscopy and electron microscopy.[104,108,116,121,123] Most authors prefer the rabbit or rat models, as experiments with primates may be hampered by cost and ethical issues. In the cat model, peeling of the ILM as a sheet, as performed in human macular surgery, is not feasible.[94]

Indocyanine green

ICG may come in contact with photoreceptors and RPE cells by passing through a MH during ICG-assisted peeling of the ILM. Several animal studies have evaluated the potential retinal toxicity of subretinal ICG injections, and most studies used ERG and histological findings to show a concentration-dependent retinal toxicity.[288] Lee et al injected subretinally ICG in various concentrations (0.6, 1.25, 2.5, or 5.0 mg/ml) in rabbit eyes. ICG at 1.25 mg/ml or higher led to degenerative changes of the photoreceptors and the RPE cells after 3 days. After 4 weeks damaged photoreceptors and outer nuclear layer were seen. In the eyes with 2.5 mg/ml or higher ICG, the photoreceptors and the outer nuclear layer were completely destroyed.[216] Similarly, Kawaji et al produced retinal detachments by injections of ICG (25, 5, and 0.5 mg/ml) into the

subretinal space of rabbit. Injections of higher concentrations of ICG caused thinning of the retina, and associated loss and apoptotic changes of the photoreceptors.[168] In addition, Penha et al evaluated the histologic and angiographic effects of subretinal injection of 0.02 ml of either iso-osmolar 0.05% ICG (279 mOsm) or hypo-osmolar ICG at 0.046% ICG (251 mOsm). Both solutions caused severe damage of all retinal layers during the entire follow-up. The damage induced by hypo-osmolar solutions was more important than that caused by the iso-osmolar solutions.[287]

Many authors have evaluated the effects of intravitreal ICG injections, and most studies found a concentration-dependent retinal toxicity associated with ICG.[72,100,229,230] In 2002, Enaida et al injected ICG solution at doses of 25 mg/ml, 2.5 mg/ml, 0.25 mg/ml or 0.025 mg/ml into the vitreous cavity of vitrectomized Brown Norway rats eyes. In the highest-dose group the retinal structure was severely deformed, and the RPE partly disappeared. No apparent pathologic change was observed by light microscopy in the low-dose groups (0.25 mg/ml or 0.025 mg/ml); however,10 days later the amplitude of dark-adapted a- and b-waves of ERGs were found to have decreased.[72] In a rabbit model, Maia et al studied the effects of intravitreal injection of 0.1 ml of ICG in three different concentrations: 0.5 mg/ml (250 mOsm), 5 mg/ml (270 mOsm), and 25 mg/ml (170 mOsm). They reported impairment of retinal function and morphology proportional to the progressively increasing ICG dosages.[229,230] Chao et al found similar results in rabbit eyes both with and without vitrectomy. Significant decrease of scotopic and photopic ERG amplitude and marked histologic changes were noted in eyes injected with 0.5 and 0.1 mg/ml of ICG in nonvitrectomized eyes. In vitrectomized eyes, decreased scotopic and photopic ERGs and mild histologic changes were noted in eyes injected with 0.5 mg/ml, but no histologic changes were noted in eyes injected with 0.1 mg/ml.[42] Grisanti et al could not find the previously described damage to the inner retina even with higher doses of ICG in a freshly enucleated pig eye.[103] Although differences within the species may contribute to these contradictory results, most studies favored dose-dependent toxicity of intravitreal ICG, as well as a role of osmolarity in dye-induced toxicity.

In 2005, Kwok et al demonstrated functional and morphological retinal damages in rabbit eyes submitted to vitrectomy, followed by intravitreal injection of 2.5 mg/ml ICG for 30 seconds with endoillumination.[203,204] Similar findings were reported by Yip et al in a rat model. Eyes submitted to intravitreal injections of 1.0 mg/ml ICG solution without illumination showed insignificant reduction in RGC density compared with the control group, whereas a significant decrease in RGC density and a significant increase in outer nuclear layer thickness was found in eyes that had ICG injection with illumination.[415] The kind and timing of illumination and its influence on toxicity deserves further investigation.

Trypan blue

In its ideal concentration for intravitreal application (from 0.06% to 0.2%), TB, revealed a low potential for retinal toxicity in animal eyes. Veckeneer et al performed gas-compression vitrectomy in rabbits and injected 0.1 ml of balanced salt solution, 0.06% TB solution, or 0.2% TB solution. Light and electron microscopic examination of the inferior retina in the 0.2% TB–treated eyes showed damaged photoreceptors and marked disorganization, whereas no histological abnormalities were found in the upper retina of the 0.2% TB–treated eyes or in any part of the retina of the 0.06% TB–treated or control eyes.[382] Grisanti et al used fresh hemisected porcine eyes and applied TB 0.15% to the posterior pole after vitreous removal. After 1 minute of exposure the eye was irrigated, filled with BSS, and illuminated with a standard surgical light at maximum power for 10 minutes. The procedure caused no histologically detectable damage when compared to the controls.[104] These results may imply that TB at concentrations of 0.15% or lower represent a safe adjuvant in vitreoretinal surgery. However, our research group showed that subretinal injection of 0.05% ICG results in more substantial retinal damage than that associated with subretinal injection of 0.15% TB. TB mainly induced RPE damage, whereas ICG induced outer nuclear layer, photoreceptor segments, and RPE damage.[287]

Triamcinolone acetonide

Various experiments using intravitreal injection of TA at concentrations varying from 4 to 30 mg yield normal morphologic and ERG findings up to 7 months post procedure .[180,314] In contrast to those reports, Yu et al injected escalating doses, from 0.5 to 20 mg, of suspended, preservative-free TA in rabbits and found prominent retinal damage at doses of 4 mg or higher.[417] These contradictory findings suggest that other factors may contribute to intravitreal retinal toxicity of TA, such as pigmentation in rabbits retinas, speed of injection, area of retina analyzed, previous vitreous detachment, or the method of TA-vehicle purification.[233,368,388]

To date, few studies have investigated the subretinal toxicity of TA. Our investigation disclosed disturbance to photoreceptor segments after subretinal injection of preservative-free TA; however, no abnormality on

fundoscopy or fluorescein angiography was observed.[231] Interestingly, Kozak et al injected 0.01 ml of 2 mg of preservative-free TA into the subretinal space in rabbits and observed hyperpigmentation in areas of the previous TA-bleb as well as histologic total absence of RPE and outer retinal cells after three months.[192] Explanations for more severe retinal damage in some studies include use of a thicker and more traumatic 32-G cannula in comparison to our 41-G, a longer follow-up period, or their method of preservative removal from TA-solution.

There is still uncertainty as to whether TA itself or the vehicle plays the more important role in retinal damage. In order to elucidate this concern, Kai et al compared TA with vehicle to preservative-free TA and demonstrated severe damage to photoreceptors only in the former.[159] In an apparently contradictory experiment, Dierks et al and Yu et al described normal retinal structure after intravitreal injection of vehicle only in animals.[65,417] Future studies should clarify if the vehicle alone, TA alone, or both chemicals in combination produce retinal damage in humans.

Newer generation stains

The safety profile of BriB in chromovitrectomy was investigated by Enaida et al in animals.[70,71] In rat and primate eyes no significant retinal pathologic changes were observed with light and electron microscopy after low-dose BriB injection. There was also no reduction in the amplitude of the ERG waves. In the rat model, Enaida et al demonstrated that BriB has good biocompatibility at the effective concentration required for ILM staining (0.5 mg/ml). However, transmission electron microscopic observations revealed that the higher-doses (10 mg/ml and 1 mg/ml) showed vacuolization in the ganglion cells and Müller cell processes in the nerve fiber layer. The potential toxicity of BriB was lower than that of the dyes widely used for ILM staining, ICG and TB, when clinical concentrations were compared. Ueno et al compared the toxicity of BriB with those of ICG and TB in a rat model of subretinal injection. ICG caused retinal degeneration and RPE cell atrophy 2 weeks after injection. TB caused less retinal degeneration, whereas BriB had no detectable toxic effects at a 2-month follow-up examination. Subretinal injections of BriB 10 mg/ml caused no remarkable histologic changes.[373] These biocompatibility tests in rat model with BriB showed that this vital dye may have lower toxicity compared to ICG and TB. These results are clinically relevant for selection of the appropriate vital dye in chromovitrectomy since the staining agent may penetrate into the MH.

At low concentration (0.2%) BroB stains the retinal surface well during vitrectomy on porcine eyes in vivo. The same result was seen in an in vivo rat model at concentrations of 0.5% and 0.02% with no signs of toxicity.[119,120,330] This dye seems to be a promising candidate for application in humans. Currently however, there are no clinical studies evaluating the toxicity of subretinal BroB.

Tthere is only one study evaluating the retinal toxicity of PB in an animal model. In 2007, our work group demonstrated that subretinal injection of PB induced less clinical and histologic damage of neurosensory retina/RPE than did TB.[232] Future animal and human studies are necessary.

Evaluation of Retinal Toxicity in In Vitro Assays

In vitro analysis of cell death may be a useful method for testing novel retinal vital stains. Animal and human retina can be exposed to test agents at a range of concentrations. Computer image analysis is then used to estimate the magnitude of the color difference between stained and unstained retina allowing evaluation of metabolic and apoptotic changes after dye exposure. The most frequently used models are human retinal pigment epithelial cells (ARPE-19), rat neurosensory retinal cells (R28), and rat retinal ganglion cells.[155,258,259]

Indocyanine green

ICG caused cytotoxicity to cultured human RPE cells,[131,132,186] retinal ganglion cells,[144,155,253] and Müller cells in a dose-and time-dependent manner in vitro.[236] Narayanan et al submitted human ARPE-19 and R28 cells to treatment with four concentrations of ICG in combination with light exposure and measured cell viability, mitochondrial function, and DNA synthesis.[258] All concentrations of ICG with light exposure caused a significant decrease in mitochondrial dehydrogenase activity, a marker for cell toxicity, in R28 and ARPE-19 cells. R28 cells did not show a significant decrease in viability. Other studies demonstrated that ICG in low concentrations (<1 mg/ml) and short incubation times (<5 min), as used in clinical practice, appear to have been well tolerated.[292] Even though ICG related toxicity is well documented, most experts agree that ICG related cytotoxic effects are influenced by osmolarity and phototoxicity.

Trypan blue

Exposure to TB in concentrations up to 0.3% in vitro on cultured human RPE and Müller cells had no toxic effect.[354] Recent investigations revealed that TB led to toxicity on cultured RPE cells at higher concentrations (above 0.5%), as indicated by the reduction in cell viability and changes in the expression of apoptosis related and cell cycle arrest genes.[208,303] Also, it seems that rat neurosensory retina (R28) cells are more

sensitive than human RPE (ARPE-19) cells to TB 0.1% with and without light exposure.[259]

Various laboratory studies compared the retina biocompatibility of TB and ICG. In one study, there was no significant difference in cytotoxicity to retinal ganglion cells between TB and ICG in short-time exposure. In long-time exposure, TB as well as ICG showed neurotoxic effect on RGCs in a dose-dependent manner.[155] In contrast to those results, another experimental set up evaluated the effects of ICG, IfCG, or TB in various concentrations on human RPE cells. After a 5-minutue exposure ICG and IfCG induced acute and chronic toxicities at a concentration above 0.05%, whereas TB evoked no acute toxicity, but was chronically cytotoxic at all tested concentrations.[186] In addition, Gale et al demonstrated that ICG causes more toxicity to human RPE cell cultures than TB, independent of any phototoxic potentiating effect or solvent toxicity.[87] This can may be explained by the fact that ICG is bound and taken up by RPE cells at concentrations lower than clinically used, compared to TB.[127]

TB's acceptance is now in question since the results of a recently reported experimental study. Authors recorded ERG from bovine retina preparations perfused with a standard solution and exposed to ICG 0.05%, TB 0.15%, and PB 0.48%. Reductions of the b-wave amplitude were found for each dye solution tested. The effects after application of PB and ICG were completely reversible within the recovery time of 30 and 60 seconds after the exposure period, respectively. The application for 15 seconds or longer of TB to the retina led to a partly reversible loss of the b-wave.[226]

Triamcinolone acetonide

Laboratory studies examined the effects of TA on various types of retinal cells, including ARPE19, human glial cells, neurosensory cells, ganglion cells or choroidal fibroblasts. A few studies have indicated that TA may promote severe toxicity to choroidal fibroblasts and RPE cells.[260,272,358,414] In addition, some investigators feel the preservative enhances the toxic effect.[41] In contrast, a few researchers found a safe in vitro profile of TA to RPE cells.[41,154] Those conflicting results highlight the importance of considereing many variables in chemical-induced retina toxicity in vitro and in the clinical setting.

Newer generation stains

Recently, Kawahara et al investigated the intracellular events in retinal glial cells exposed to ICG at 0.25 mg/ml and 2.5 mg/ml in comparison to BriB G at 0.25 mg/ml. Transmission electron microscopy revealed apoptotic changes of the caspase cascade only in the ICG-treated cells.[167]

Mennel et al used an in vitro model of the outer blood–retinal barrier (BRB) consisting of human RPE cells and choroidal endothelial cells cultured in monolayers on semipermeable membranes to evaluate the influence of ICG (5 mg/ml, 0.5 mg/ml, 0.125 mg/ml), TB (1.5 mg/ml, 0.15 mg/ml), and PB (2.4 mg/ml, 0.24 mg/ml) on BRB function. By measurement of the transepithelial electrical resistance (TER) the stable barrier function was determined. After application of TB, PB, and the lowest concentration of ICG of 0.125 mg/ml, the TER remained stable in both models. In contrast, ICG in the two other concentrations reduced the function of the outer BRB.[247]

In 2005, Haritoglou et al evaluated the staining characteristics and safety of six potential new dyes for intraocular surgery: light green SF (LGSF) yellowish, E68, bromophenol BroB, Chicago blue (CB), rhodamine 6 G, rhodulinblau-basic 3 (RDB-B3), all in balanced salt solution in concentrations of 0.2% and 0.02%. Rhodamine G6 and RDB-B3 showed adverse effects on ARPE-19 cell proliferation at a concentration of 0.2% and were excluded from further investigation in primary RPE cells. The remaining four dyes showed no toxic effect on ARPE-19 and primary RPE cell proliferation at concentrations of 0.2% and 0.02%. Cell viability was affected by LGSF yellowish (0.2%) and CB (0.2% and 0.02%). Two dyes (E68 and BPB) showed no relevant toxicity in vitro.[120]

Jackson et al investigated retinal vital stains (alcian blue; diethyloxadicarbocyanine; Evans blue; Fast green; fluorescein; JG; MB; naphthol green; neutral red; procian yellow; rose bengal; and TB) for their potential surgical utility in bovine retinas and found that five agents showed favorable staining characteristics—Evans blue, rose bengal, naphthol green, neutral red, and TB. Safety testing of these five agents did not show toxicity, except on glial cells exposed to rose bengal.[148]

Luke et al recently released their data on the toxicity of BriB in a model of isolated, perfused vertebrate retina. After applying BriB at a concentration of 0.25 mg/ml epiretinally for 10–120 seconds, they found reductions of the a- and b-wave amplitude, but these were rapidly and completely reversible. No differences were found between the ERG amplitudes before and after dye application at the end of the washout, indicating that BriB at the commercially available concentration is a safe staining agent for the retina.[225]

Type of Cellular Injury after Retinal Exposure to Dyes

Drug-induced cytoskeletal alterations may be a useful safety-screening marker for retinal and lens

toxicity.[384] Actin filaments play a critical role in the normal physiology of lenticular and retinal cells. In RPE cells, actin microfilaments are essential for correct internalization and phagocytosis of outer segments, and the microfilaments are a key component of microvilli protrusions that surround rod outer segments.[77,102,199] Cell calcein esterase activity, cell morphology (viability), mitochondrial function, and cell proliferation/DNA synthesis are other parameters. ICG decreases the mitochondrial dehydrogenase activity and increases the DNA synthesis in retinal cells, markers for cell toxicity and dysfunction.[258]

Kunikata et al showed that hypothermia can reduce toxic damage to RPE cells after exposure to TB. At 37 °C, TB 0.5% and 0.05% reduced the number of viable RPE cells, whereas at 8 °C, TB 0.05% was not toxic.[196]

Another proposed mechanism to explain types of cellular injury is activation of apoptosis—the process of active cellular self-destruction that requires the expression of specific genes. One of them, bcl-2 (B-cell leukemia/lymphoma-2) is the prototypic member of a family of cell death regulatory proteins and is found mainly in the mitochondria.[328] bcl-2 is up-regulated in the early stage of neurotoxin-induced neuronal degeneration.[6] Exposure to 0.5% ICG increases the expression of bcl-2 mRNA in RGCs, which may suggest that bcl-2 is transiently up-regulated in response to the insults by cytotoxins, including ICG. Because up-regulation of bcl-2 by ICG corresponds to RGC death, ICG may initiate RGC death, at least in part, through an apoptotic pathway.[249]

Mechanisms of Dye-induced Retinal Toxicity

The subretinal injection of dyes may be associated with a greater risk of toxicity than intravitreal injection.[128,168,216] This might be because the presence of Müller glial cells and their basement membrane (ILM) between the vitreous dyes and the neural cells has a blocking effect.[306] Furthermore, because photoreceptors have higher metabolic rates and contribute to the maintenance of visual pigment recycling, they could be more sensitive to external stimuli that cause apoptosis.[130] Generally, dyes exacerbate phototoxicity secondary to surgical illumination. Light damage to the retina occurs through thermal, mechanical, or photochemical effects. The particular mechanism activated depends on the wavelength, intensity, and duration of the injuring light. The various light damage mechanisms may overlap. Strongly absorbing tissue components will tend to "concentrate" the light energy.[98] The duration of light has been proven to be a significant factor in ICG toxicity.[309]

There is strong evidence for dye-induced, dose-dependent toxicity to retinal cells. Postulated mechanisms of intravitreal dye-related toxicity include surgical damage to superficial retinal cells, ion-related damage, osmolarity effect of ICG solution on the vitreoretinal interface, and light toxicity.[306,309,372]

Surgery-related damage to the nerve fiber layer

Visual field defects as a complication after vitrectomy for MH surgery vary in different clinical series from 1% up to 70%.[286] Proposed mechanisms include mechanical trauma to the optic nerve head, disturbance of the chorioretinal circulation, and dehydration injury of the nerve fiber layer during fluid–gas exchange.[26,242] However, the different localizations of visual field defects suggests different pathogenic mechanisms. Haritoglou et al found that the incidence of visual field defects increased when they began using ICG. They felt this was caused by an abnormal cleavage plane and damage to the innermost retinal layers.[110,115] Uemura et al also reported the nasal or concentric visual field defects in four of seven eyes with the use of ICG-staining in the treatment of ERM. They speculated that the ILM was not stained by ICG in the macular area because the ERM protected this area from ICG.[372]

Kwok et al hypothesized that ICG-assisted ILM peeling traumatizes the superficial retinal vessels and reported 2 cases (3.6%) of vision-threatening vitreous hemorrhage in a series of 55 cases.[206] They suspected that ILM peeling in hypertensive patients triggers vitreous hemorrhage in the already damaged superficial retinal vessels.

Damage induced by secondary ions within the dye solution

The iodine present in the ICG solution may be responsible for the observed retinal toxicity. Iodine and its derivative compounds such as sodium iodate are highly toxic and a single injection leads to a necrosis of practically the complete RPE.[10,78,139] High dose topical or intraocular iodine induces severe corneal and retinal damage. Whitacre et al demonstrated toxicity and intraocular inflammation after intravitreal injection of povidone-iodine at concentrations above 0.5% in a rabbit model.[398] Full thickness retinal necrosis and a profound, lasting reduction in the ERG were produced in all of these eyes. ICG is a synthesized without sodium iodine may prevent this toxicity.

Ho et al implicated sodium (Na+) in RPE toxicity after ICG use for MH surgery.[131] Removal of Na+ from the solvent reduced ICG-induced RPE toxicity perhaps because Na+ reduced uptake of ICG. Sodium removal reduced the ICG-induced

changes in cell morphology and improved RPE cell viability. The Na+ free BSS solution replaced NaCl, Na_2HPO_4, and $NaHCO_3$ with choline chloride, K_2HPO_4, and $KHCO_3$. This reconstitution method may allow safer intravitreal use of ICG in MH surgery.

Damage induced by osmolarity of solution

Retinal toxicity secondary to changes in osmolarity at the vitreomacular interface was first reported by Marmor et al in 1979.[235] They described cellular damage at the vitreoretinal interface, including nonspecific shrinkage and disruption of the cellular architecture caused by induction of osmotic levels of >500 mOsm. Because differences in osmolarities between the subretinal space and choroid are corrected rapidly by the adjacent tissues, the osmolarity of a subretinal solution can result in retinal and RPE damage.[81,147,262–264]

The presence of sodium iodine in the ICG solution requires dilution in water, thereby resulting in a hypotonic solution of 248–275 mmol/kg. Intravitreal dye injections may rapidly change the osmolarity in the vitreous cavity. In vivo and in vitro studies indicate that hypoosmotic ICG solutions harm the RPE, and this effect may be amplified by additional intra-operative light exposure.[89,91,132,143,144,407] Sippy et al first reported that hypo-osmotic ICG solution plus light promoted a marked reduction in enzymatic activity in RPE cells.[344] Later, Stalmans et al found a correlation between severity of damage to RPE cells and duration of ICG incubation, light exposure time, and osmolarity of the solvent.[355] Other studies reveal a correlation between functional outcome and osmolarity, with those using iso-osmolar solutions generally reporting better results.[91,111] One exception was Kandonosono et al, who obtained good results with a hypo-osmolar solution (270 mOsm), but this had the possible protective effect of hiperviscosity.[158]

Light-mediated damage

The overlap of ICG absorption spectra with different types of endoillumination for vitreoretinal surgery may pose the risk of phototoxicity to the retina. Commercially available light sources for endoillumination during vitrectomy emit a wide range of light wavelengths from short (380 nm) to long (830 nm), whereas the light absorption of ICG remains in the near-infrared part in a 780- to 830-nm spectrum.[213] The characteristics of ICG absorption spectra of the retina have been evaluated in human donor eyes by diffuse reflection spectroscopy.[109,116–118,402,403] ICG may change the absorption spectra of the retina, and this effect is dependent on the concentration and volume applied.[109,116,118]

Intravitreous ICG along the ILM may enhance light absorption and increase the local temperature on the retina, the so-called photooxidation type I.[351] Indeed, photooxidation type I with ICG has been reported in the treatment of choroidal neovascularization using photodiode laser.[302] In 2001, a modified technique in which intravenous ICG led to photodynamic effects or photooxidation type II in choroidal neovascularization was described.[51] As ICG-induced photodynamic lesions occur only in the presence of high oxygen concentrations such as occur with choroidal neovascularization, it is unlikely that photooxidation type II plays a significant role in the low oxygen concentration within the vitreous. Even though ICG has been shown to penetrate deeper retinal layers, it has not yet been isolated in the choroid or choriocapillaris.

Gandorfer et al studied the histologic effect of 0.05% ICG in human donor eyes exposed to different types and wavelengths of light. In this study, exposure of ICG-stained ILM to wavelengths of >620 nm led to severe damage to the inner retina. ICG staining alone or in combination with wavelengths ranging from 380 nm to 620 nm demonstrated only rupture of Müller cells and partially detached ILM.[89,91] Grisanti et al repeated this study by using fresh pig eyes and found no histologic damage with exposure of the retina to different concentrations of ICG. They concluded that even though differences within the species may contribute to these contradictory results, the vitality of the tissue may have influenced the outcome in this ex vivo system.[99] One recent study on enucleated human and porcine eyes found that disorganization of the innermost retina and ILM loss were more severe in eyes exposed to a halogen than a xenon light source.[117]

In vivo studies have evaluated the effect of ICG plus endoillumination on retinal tissue. Maia et al investigated the toxic effects of subretinal and epiretinal ICG injection plus light exposure on rabbit eyes. No histologic damage was observed with epiretinal ICG injection, but severe retinal damage occurred with subretinal application. The toxic changes were similar with or without light exposure.[229,230] These animal investigations on ICG interaction with light may not quite mimic the human eye. The animals do not possess maculas similar to those of humans and young animals with healthy RPE were used. In addition, risks from light exposure are not limited to the intraoperative, because ICG may persist for several months after surgery, and environmental light could negatively interact with the dye. Further studies should focus on experimental methods similar to the clinical setting and consider different types of light sources and time of exposure.

The Use of Vital Dyes in Glaucoma Surgery

RATIONALE

The main application of biological stains in glaucoma surgery is to evaluate the patency and leakage of glaucoma filtering blebs. In addition, anti-metabolic agents used during trabeculectomy may be colored with vital dyes to allow better control of their placement in ocular tissues.

INTRA-OPERATIVE STAINING ANTI-PROLIFERATIVE AGENTS FOR TRABECULECTOMY AND BLEB NEEDLING

Trypan Blue

TB may be applied to determine the patency of previous filtering surgery during phacoemulsification. Agrawal et al performed phacoemulsification and TB-assisted anterior capsulorhexis in 15 patients with trabeculectomy blebs. Concentration 0.06% TB was injected intra-operatively into the anterior chamber to facilitate the capsulorhexis. In 14 cases the dye was transmitted into the bleb area, and after 24 hours no trace was found.[2] Dada et al described a similar case of inadvertent staining of the bleb with TB during phacoemulsification. TB used to stain the anterior capsule thus allowed the visualization of the drainage function during cataract surgery.[56]

Pharmacologic adjuvants such as mitomycin C (MMC) of 5-flurouracil (5-FU) are transparent substances that must be precisely placed during glaucoma filtering surgery. Healey et al examined the utility of using TB to color anti-metabolic agents and the effect of TB on anti-metabolite cytotoxicity in vitro. The addition of 0.05% TB to MMC did not alter MMC-induced cell death or the number of viable fibroblasts in vitro. 0.1% TB was added to MMC and 5-FU in final concentrations between 0.01% and 0.05%. The mixture was applied to Tenon's capsule and sclera on sponges for 3 minutes or by direct subconjunctival injection after completion of surgery. Clinically, TB clearly delineated the anti-metabolite treatment area and facilitated control of excess anti-metabolite at the wound margins as well as sponge removal. Any leakage from the injection site could be easily seen. No adverse effects attributable to TB were found in 2 years of follow up.[123]

Indocyanine Green

Okasaki et al used ICG to visualize bleb leakage during trabeculectomy. A 0.25% ICG solution was applied over the bleb including the conjunctival wound at the end of the surgery. If any bleb leakage were present, it could be repaired intraoperatively. No toxicity to conjunctiva or cornea was observed.[275] Another study used ICG during the removal of an overhanging filtering bleb after trabeculectomy. During the initial stage of the surgery, a 0.25% ICG injection was placed into the overhanging portion of the bleb. It was then possible to safely separate the original bleb without injuring its thin surface.[145]

ICG toxicity to corneal endothelium and retina is dose-dependent. When used for glaucoma surgery, ICG must be applied carefully to avoid dye penetration into the bleb and anterior chamber.[145,275]

Reeves et al compared the effects of ICG with and without MMC on proliferation of cultured human Tenon fibroblasts.[301] The MMC treatment alone resulted in a significant reduction in viable fibroblast number, but ICG at concentrations of 0.5% and below alone or in combination with MMC did not significantly alter the cell numbers compared to MMC alone.

Fluorescein

Matsuo et al performed a case-controlled study to evaluate intraocular penetration of topical fluorescein in eyes with avascular blebs after trabeculectomy.[237] They included patients with open-angle glaucoma and functioning avascular blebs, eyes with open-angle glaucoma treated with topical medications and no previous surgery, and untreated eyes suspected of having open-angle glaucoma. The fluorescein concentration in the superior peripheral and central corneal stroma and anterior chamber was determined 30 and 60 minutes after the instillation. The dye concentration in the superior cornea was significantly higher in eyes with blebs or those treated topically compared with untreated eyes. The concentration in the anterior chamber was much higher in eyes with blebs than in those that were untreated or treated topically.

The Use of Vital Dyes for Orbit Surgery

RATIONALE

Only a few reports address the application of TB and MB for tissue visualization in orbital surgery. Vital staining in allows intra-operative identification of various epibulbar tissue layers during enucleation, visualization of dermoid cysts and tumors, and visualization of intraorbital fat compartments.

VITAL DYES FOR IDENTIFICATION OF EXTRA-OCULAR TISSUES DURING ENUCLEATION

TB has been used to stain Tenon capsule during enucleation surgery. The tissue layers sutured over

the implant consist of the posterior Tenon capsule, anterior Tenon capsule, and the conjunctiva. However, in some patients it can be difficult to distinguish them accurately. TB also provides an excellent means by which surgeons in training can be certain that closure of the wound occurs in separate layers. Prior to closure of the anterior Tenon capsule and conjunctiva, TB can be instilled into the wound with a syringe. The wound is then irrigated with normal saline. The Tenon capsule clearly stains.[45] Identification and closure of the Tenon capsule and conjunctiva in separate layers may prevent formation of conjunctival cysts, particularly in cases of secondary implants with previous disruption of the anatomy.[185,347] Ensuring closure of the anterior Tenon capsule and conjunctiva as separate layers may also reduce other complications such as implant exposure and/or extrusion.[338]

VITAL DYES FOR REMOVAL OF ORBIT TUMORS AND CYSTS

MB guides excision in Mohs' micrographic surgery (MMS), used for the precise microscopic removal of certain cancers. In the orbit the vital dye MB may be used to identify incomplete specimen removal and thus facilitate MMS technique. This vital dye-enhanced procedure may minimize the chance of regrowth and lessen the potential for scarring or disfigurement.[418]

Pre-operative MB injection may facilitate removal of orbital dermoid cysts, common ocular choristomas that result from sequestration of surface ectoderm during early development.[250] During surgical resection, accidental rupture of the cyst may occur, causing marked inflammation and increasing the risk of recurrence. Injection of the cyst with MB stains the cyst wall. This enhances the visibility of the cyst in the surgical field, minimizing the risk of an inadvertent rupture, especially in the removal of recurrent or complicated dermoid cysts. In case of rupture of the cyst before surgery or when inadvertent rupture occurs intra-operatively, MB injection can also be used. Care should be taken to avoid spillage of MB and staining of surrounding tissue.[369]

VITAL DYES FOR IDENTIFICATION OF INTRAORBITAL FAT COMPARTMENTS

Resection of fat compartments is common in upper lid blepharoplasty. There are two fat compartments in the upper eyelid (the central and the medial) and three fat compartments in the lower eyelid (the medial, the central and the lateral). A third accessory or ectopic fat pocket in the upper eyelid is frequently present.[291] Injection of MB vital dye into a fat compartment may facilitate visualization of the adipose pocket.[18]

The Use of Vital Dyes in Strabismus Surgery

Tenectomy of the superior oblique tendon may be challenging because of difficulty visualizing the small anatomic structures. Staining Tenon's fascia and muscles during strabismus surgery may assist the surgeon. Saxena et al described the use of vital dyes for staining the superior oblique tendon in 15 cases using either TB, GV, or ICG.[324] They found TB and ICG to be better agents for staining the tendon's fascia, with no staining of the scleral tissue. No complications have been reported with TB-guided tenon coloring. Further investigations should evaluate if other periocular tissues can be stained during strabismus surgery.

Conclusions and Final Remarks

There is general agreement that, in cataract surgery, vital dyes enable much better visualization of the anterior capsule, although some issues remain. We reviewed 60 studies addressing the use of vital dyes in cataract surgery, to stain anterior and posterior capsule and visualize the anterior vitreous. Table 1 summarizes these articles, separating them into human and animal studies. TB was the most studied dye, although both TB and ICG effectively stain the anterior capsule. TB has been found overall to be safe, and concentrations lower than 0.06% may provide a contrast between the stained capsule and the underlying lens. Injection of TB under an air bubble stains the anterior capsule with no corneal toxicity, even in long term follow-up. ICG injected under air bubble appears to non-toxic to cornea; however, long-term follow-up is needed. ICG use in cataract surgery raises the procedure's cost when compared to TB. Alternative dyes such GV, BriB, MB, and autologous blood have not yet proved their safety to the anterior segment. For vitreous identification TA is the first-line coloring agent. Further studies are needed to establish the staining technique, concentration, and comparison with TB and ICG.

For vitreoretinal surgery vital dyes allowed much better identification of the semi-transparent retinal tissues. ICG, IfCG, BriB, and BroB may be the best stains for the ILM, whereas for the ERM, TB and PB may be preferred. The high water content of the vitreous means that many dyes, such as TB, ICG, or PB, may stain the vitreous well. An outstanding agent for vitreous visualization is the crystalline steroid TA.

In regard to the toxicity issues in chromovitrectomy, some preliminary conclusions may be drawn to date. First, every vital dye injected intravitreally has a dose-dependent toxicity to the retinal tissue. ICG or IfCG should be injected in concentrations below 0.05%. TB should be injected in low concentrations such as 0.06%. In addition, there is strong evidence that light exposure, osmolarity, and the presence of ions such as Na+ and iodine may further damage to the retina. Recommendations include a very low amount of dye injection onto the pre-retinal membrane, avoidance of long macular exposure to endoillumination, and removal of sodium and iodine from staining solutions by applying IfCG instead of ICG and diluting the dye in glucose 5%. Osmolarity must be taken in consideration when vital dyes are injected intravitreally. Dyes provided as powders, such as ICG or IfCG, should be diluted in glucose 5% rather than water. In ocular surgery TB in low concentrations such as 0.02% may be used in order to facilitate stromal dissection or promote close alignment of both the edge of host and donor DM in keratoplasy. For intra-operative application of vital dyes in glaucoma strabismus,and in orbit and conjunctival surgery, more detailed examination is necessary to elucidate their precise indication and safety in ocular surgery, but preliminary data indicate that TB may may be useful to determine the patency of the filtering surgery or to color anti-metabolites, whereas MB may assist in identification of orbit dermoid cysts or adipose tissue. In the conjunctiva TB may enable precise localization of the size of neoplastic lesions. Vital dyes facilitate identification of the fine, semi-transparent ocular structures.

Method of Literature Search

The Medline Web site (www.ncbi.nlm.nih.gov/PubMed/) and ISI (from 1986–2008) databases were accessed for the period of 1998 through 2007, and searched for relevant information related to the topic the use of vital dyes in ocular surgery using the key words: *indocyanine green, trypan blue, patent blue, brilliant blue, bromophenol blue, infracyanine green, triamcinolone acetonide, retina, anterior capsule, capsulorrhexis, strabismus, cataract, cornea, keratoplasty, orbit surgery, conjunctiva, internal limiting membrane, macular hole, peeling, chromovitrectomy, macula surgery, epiretinal membrane, ILM.* Articles cited in those references were included if found to be relevant, and those found elsewhere and considered to be important for the topic were also included in this review. Criteria of inclusion of articles from other sources were the present clinical value or the original importance of the article to the particular subject of the manuscript. All articles in English, German, Portuguese, Spanish, and Italian were reviewed as full-text in its original language without translation. For articles in languages other than those previously mentioned, abstracts were evaluated and, when appropriate. included as well.

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