Theme Article

Vital Stains in Retina and Vitreous

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Abstract *Dyes used to stain living tissues are known as vital dyes and these have become an effective surgical aid in ocular tissue identification and visualization in ophthalmology. "Chromovitrectomy" is a phrase used for describing the use of vital dyes to stain these transparent tissues and facilitate their manipulation during vitreo-retinal surgery.1 Common vital dyes used in the posterior segment are indocyanine green (ICG) and brilliant blue (BB); and dyes which are still under research include patent blue, bromophenol blue, light green, and Evans's blue.*

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Dyes used to stain living tissues are known as vital dyes and these have become an effective surgical aid in ocular tissue identification and visualization in ophthalmology. "Chromovitrectomy" is a phrase used for describing the use of vital dyes to stain these transparent tissues and facilitate their manipulation during vitreo-retinal surgery.[1]

Common Vital Stains

These may be classified according to the chemical composition: (1) azo dyes like trypan blue; (2) arylmethane dyes like brilliant blue; (3) cyanine dyes like Indocyanine green; (4) xanthene dyes like Fluorescein; and (5) colored corticosteroids like Triamcinolone acetonide.[2]

1. Trypan Blue (TB)

Concentration: 1.2mg/ml (0.15%)

Brand Name: Membrane Blue (DORC International, Zuidland, Netherlands).

Mechanism: TB has high affinity for cellular-proliferative tissues, hence, it stains the ERM very well but not the ILM.[1] To augment staining property, it can be injected after air-fluid exchange or mixed with 5-10% glucose. It may cause chronic retinal toxicity by inducing arrest of the cell cycle at G0–G1 via increased expression of p21.[2]

2. Brilliant Blue (BB)

Concentration: 0.025%
Brand Name: Brilliant Peel (Geuder, Heidelberg, Germany)
Mechanism: BB markedly stains ILM without staining the epiretinal membrane or the vitreous.[3]
It is hydrosoluble; it would thus penetrate less into the cells and be more easily washed away, leaving less residues after surgery.[2] It is hence considered to have to have lower toxicity.

3. Indocyanine Green[4]

Concentration: 5 mg (0.5%); 25 mg (2.5%); 50 mg (5.0%)
Brand Names: ICG (Pulsion Medical Systems, Munich, Germany) ICV Indocianina Verde (Ophthalmos, São Paulo, Brazil), Diagnogreen (Daiichi Pharmaceutical, Tokyo, Japan), and IC-Green (Akorn, Buffalo Grove, USA).

Mechanism: It has a maximum affinity for laminin and collagen type 4 (which is found in the basement membrane) of the internal limiting membrane (ILM), due to which it was most widely used to stain the ILM during vitreoretinal surgeries.

Uses:

- To peel the ILM for macular hole treatment
- To help ILM peeling in other diseases, such as diabetic macular edema
- for better visualization of epiretinal membranes (ERMs) in vitrectomy for proliferative diabetic vitreoretinopathy, idiopathic ERMs, and proliferative vitreoretinopathy.[2]

Disadvantages

- ICG has been found to be associated with the risk of damage to the photoreceptors and RPE cells, RPE atrophy, visual field defect, loss of epiretinal cellular integrity, and optic nerve damage. However, in low doses, it is considered safe.
- For use in anterior capsule staining in cataract surgery, the dye has to be reconstituted and diluted. This has to be folowed by filtration to prevent undissolved particles from entering the eye before use.
- Use in intraocular surgery is not approved by the FDA.

4. Infracyanine Green (IfCG)

Concentration: 5 mg (0.5%) and 25 mg (2.5%) of infracyanine green
Brand Name: Infracyanine (Laboratoires SERB, Paris, France).

Mechanism: IfCG also binds with high affinity to the acellular ILM and facilitates its visualization and peeling similar to ICG.5 Its advantage over ICG is that it is synthesized without sodium iodine, as it is believed that iodine damages the retina. However, IfCG can be phagocytosed by RPE cells, remaining in the interior of these cells for long periods, with a risk of inducing chronic toxicity. The downside of being iodine free is that it is not water soluble and has to be dissolved in a 5% glucose solvent.

5. Sodium Fluorescein

5-25% of fluorescein dye is commonly used during fluorescein angiography. The clear vitreous can be stained markedly green by SF administered 12–16 h before surgery and new research involves the use of intraoperative three-dimensional fluorescein angiography (3D-FA)-guided pars plana vitrectomy.6

6. Triamcinolone Acetonide (TA)

Concentration: 0.1- 0.3ml of 40mg/ml (4%)
Mechanism: Triamcinolone acetonide (TA) is a synthetic

insoluble corticosteroid.[1] Its crystals have an affinity for acellular tissues such as vitreous and internal limiting membrane – they deposit on the vitreous gel and helps in easy differentiation between vitreous free area from an area where vitreous is still present, facilitating complete vitrectomy.

Uses:

- During vitrectomy, it facilitates removal of posterior hyaloid from retina and decreases proliferative vitreoretinopathy.
- Injecting this steroid during vitrectomy for the management of retinal detachment may prevent fibrin reaction and PVR postoperatively
- Other than as a dye, intravitreal triamcinolone is FDA approved (Triesence) for treatment of macular edema and uveitis.[7]

Disadvantages

- It may remain in the vitreous cavity even upto 40 days after injection.
- Risk of formation of cataract.
- Risk of IOP spike
- The commonly used formulation of TA, kenalog, is not formulated for the eye, for this reason, there is a risk of pseudoendothalmitis and retina toxicity when injected intravitreally.[2]

7. Fluoromethonolone Acetate

Studies have shown that FMA can be used as an alternate to TA as it doesn't show any abnormal changes in ERG as well as no histological changes. However, not enough studies have been conducted to compare advantages and disadvantages of newly discovered vital dyes and their long-term effects.[8]

Dyes Under Research

1. Bromophenol Blue: It is a novel adjunct used in Concentration of 0.13% to 0.2% and stains the epiretinal membrane, internal limiting membrane and vitreous well. Literature differs regarding toxicity of the dye, but it is still not FDA approved for intraocular use.[3]

2. Patent Blue: Patent Blue (0.25%) has recently been discovered that to stain the glial ERM noticeably with poor staining of ILM. It causes retinal toxicity at higher doses, and is not FDA approved for intra-ocular use.[9]

3. Anthocyanins: it is a natural dye derived from the acai fruit. It is seen to stain posterior hyaloid and ILM in studies conducted with animals, with no toxicity to the retina.[10]

4. **Trisodium, Orangell and Methyl Violet** – novel dyes to stain vitreous and preretinal tissues without major toxicity concerns.[11]

Commercial Combinations

- **ILM Blue (DORC) -** BBG (0.25 mg/ml) with 4% polyethylene glycol. It is a heavy dye facilitating sedimentation on the retina.[12]
- **MembraneBlue-Dual (DORC) -** Another heavy dye containing trypan blue 0.15%, BBG 0.025%, and 4% polyethylene glycol. It is suitable for ILM, ERM, and PVR membrane staining. [12]
- **Lutein:** lutein + zeaxanthin 0.3% + BBG 0.025%.13 It is another heavy dye that also helps in better view of vitreous and posterior hyaloid. Lutein and xanthin do not affect the staining property of BBG on the ILM, and protect the retina by filtering blue light.

Dye injection techniques

- The "dry method" or "air-filled technique" – in which the fluid in the vitreous cavity is removed by a fluid-gas exchange before dye injection. This has the advantage of concentrating the dye in the posterior pole and avoiding contact at the posterior capsule of the lens, but it may expose the retinal surface to a higher concentration of dye. [2]
- The "wet method" or "fluid-filled technique." - In this approach, the intravitreal fluid (usually BSS) is left inside, while the dye is injected. The amount of dye in contact with the retinal surface becomes much lower because it is immediately washed out by the fluid in the vitreous cavity. Hence this method is safer and faster during surgery than dry method.[2]
- Double staining – It is used for peeling of ERM and ILM. After vitrectomy, the ERM is first stained with BBG, and then peeled. BBG stain is then reapplied, and the residual ILM was peeled.

Other Uses Of The Vital Stains In Ophthalmology

1. Fluorescein

- Endothelial cell viability
- To see extent of epithelial defect in the cornea, especially in ulcers
- Anterior capsule lens identification during cataract surgery

2. Trypan Blue

- Endothelial cell viability (0.001-0.1%)
- Identify anterior lens capsule during cataract surgery (0.06%)
- Keratoplasty (0.02% - to stain DM of donor and recipient cornea)
- Conjunctival cyst capsule identification
- To identify clear corneal incision with dye coated blade
- Visualization of drainage function during cataract surgery in a operated trabeculectomy eye (0.06%)
- Staining of SO in strabismus surgery
- Enucleation- to stain tenon's capsule

3. Triamcinolone

If a posterior capsular rent occurs during cataract surgery, triamcinolone can be used to know if any vitreous strands are left in AC [anterior chamber] after anterior vitrectomy.

4. ICG

- Endothelial cell viability (0.5% for 3 min)
- Conjunctival cyst capsule identification
- Anterior lens capsule in cataract surgery (0.125-0.5%)

SUMMARY

Substance	Dose	Molecular Weight	Dilution	Affinity	Preventing Retinal Toxicity
Triamcinolone acetonide	40mg/ ml (4%)	434 Dalton	no	vitreous	Using preservative-free solution
Indocyanine green	5mg (0.5%) 25mg (2.5%) 50mg (5%)	775 Dalton 3-5% iodine	(0.05%) dissolve in small amount, use BSS for dilution	ILM	Add 1ml water to 1 vial of 5 mg, take 0.1ml of it and mix with 0.9ml BSS
Infracyanine green	5mg (0.5%) 25mg (2.5%)	775 Dalton Same as ICG, but no sodium, iodine	(0.05%) dissolve in 5% glucose	ILM	Add 1-2ml of 5% glucose to 1 vial of 5mg
Trypan blue	1.2mg/ ml (0.15%)	960 Dalton	no	ERM	Mix 0.3ml with 0.1ml of 5% glucose
Brilliant blue	0.25mg/ ml (0.025%)	854 Dalton	no	ILM	Use with dilution
Patent blue	2.5mg/ ml (0.25%)	582 Dalton	no	ERM	Mix 0.3ml with 0.1ml of 5% glucose

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