Vital Dyes For Chromovitrectomy: Colours for the Vitreoretinal Surgeon!!!

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Chromovitrectomy refers to the application of vital dyes during retinal surgery to visualize preretinal tissues and membranes. Chromovitrectomy arises from the difficulty in visualizing and removing the several thin and transparent tissues in the vitreoretinal interface including the internal limiting membrane (ILM), epiretinal membrane (ERM) and the vitreous. These tissues are involved in the pathogenesis of several macular disorders including macular holes and diabetic macular oedema. Surgical manipulation of these poorly visualized tissues have been shown to induce gliosis, iatrogenic chorioretinopathy and phototoxicity. Staining of these tissues with vital dyes may improve their visibility, enhance the ability to peel them as well as ensure complete removal of all tissues, which may lead to a better visual result postoperatively with a lesser recurrence rate.

An ideal vital dye for chromovitrectomy should have the “ability to selectively stain” the internal limiting membrane and the epiretinal membrane, leaving the retina unstained. It should provide adequate colour difference between the stained ILM/ERM and the normal retina. Other favorable characteristics are 1) rapid elimination from vitreous cavity 2) photochemical stability 3) solubility in balanced salt solution 4) absence of toxicity and 5) an adequate light absorption profile.

The vitreoretinal interface staining agents have been used since 2000. They can be classified into three generations depending on the time of introduction.

First Generation: (2000): Indocyanine Green (ICG)
Second Generation: (2003): Infracyanine Green (IFCG), Trypan Blue (TB) and Triamcinolone acetonide (TA)

Table 1 gives the comparison of staining characteristics and structures of various dyes used for chromovitrectomy.

**Indocyanine Green (ICG)**

ICG is a tricarbocyanine anionic dye with a molecular formula of C43H47N2NaO6S2 and a molecular weight of 775 Daltons. This green dye has amphiphilic properties and hence interacts biochemically with different human tissues. ICG demonstrates greatest affinity to the extracellular matrix components of the ILM, thereby exhibiting an ability to selectivity stain the ILM.

Chromovitrectomy using ICG for dye assisted peeling of ILM gained acceptance for the management of macular holes. Its use was later extended to improve visualisation of the glial ERM, proliferative membranes of proliferative diabetic retinopathy (PDR) and proliferative vitreoretinopathy (PVR).

Controversial reports on the toxic effects of ICG have been published. These included Muller cell, RPE damage; visual field defects and optic atrophy.
Histopathology of the peeled ILM after ICG assisted ILM peel during chromovitrectomy revealed the presence of cellular structures on and under the ILM\textsuperscript{19,20}. Detection of the presence of retinal elements (plasma membrane of Mullers cells, myofibrocytes, astrocytes) on the peeled ILM raised the issue of retinal damage during peeling. Animal studies and in-vitro experiments indicated a dose dependent ICG mediated toxicity to retinal elements (Mullers cells, ganglion cells, photoreceptors and RPE)\textsuperscript{11,21-24}. The hypotonic ICG solution has been shown to cause osmotic damage to the retinal cells.

Exposure to ICG causes damage to the photoreceptors and RPE cells leading on to apoptosis or necrosis due to light induced damage\textsuperscript{25-27}. Subretinal injection of ICG in rabbit models may result in RPE damage even in concentration as low as 0.5 mg/ml.

There is paucity of published data comparing the effect of ILM peeling with and without the use of ICG.

Majority of studies (Table 2) used ICG in higher concentration and this factor could be responsible for the toxicity. We suggest three safety measures when using ICG for ILM peeling.

1. Perform a fast surgical procedure in order to minimize the duration of contact with RPE cells and to minimize the ICG exposure to light from endo illuminator.
2. Use ICG concentrations lower than 0.5 mg/ml to minimize the risk of RPE damage and possible retinal toxicity.
3. Avoid ICG injection direct through the macular hole by any method to control ILM staining (slow injection, use of the 20 gauge, prototype painting brush called vitreo retinal internal limiting membrane color enhancer (VINCE)\textsuperscript{31}, or by use of perfluorocarbons over the macular hole etc).

**InfraCyanine Green (IfCG)**

IfCG possesses two well recognized pharmacological differences from ICG, which vouches for its safe profile in chromovitrectomy\textsuperscript{32,35}.

1. IfCG is produced in a synthesis mode without sodium iodine. High dose of topical or intraocular iodine can induce severe corneal and retinal damage.
2. The presence of sodium iodine in the ICG solution requires dilution in water resulting in a hypotonic.

### Table 1. Properties of dyes used for chromo vitrectomy

<table>
<thead>
<tr>
<th>Molecular formula</th>
<th>SF</th>
<th>ICG</th>
<th>IfCG</th>
<th>TB</th>
<th>TA</th>
<th>PB</th>
<th>BrB</th>
<th>FMA</th>
<th>BBG</th>
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<tbody>
<tr>
<td>C₁₀H₁₀Na₂O₅</td>
<td>376</td>
<td>774</td>
<td>774</td>
<td>961</td>
<td>434</td>
<td>582</td>
<td>670</td>
<td>418</td>
<td>854</td>
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<tr>
<td>C₁₁H₁₇N₂NaO₅S₂</td>
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<tr>
<td>C₁₄H₂₄Na₄O₁₄S₄</td>
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<td>C₂₄H₃₃S₂O₅</td>
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<tr>
<td>C₇H₅Na₃S₂O₅Na</td>
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<tr>
<td>Chemical Group</td>
<td>Xanthene</td>
<td>Tricarcyanine</td>
<td>Tricarcyanine</td>
<td>Diazocyanine</td>
<td>Long-acting Steroid</td>
<td>Triyl methene</td>
<td>Triyl methene</td>
<td>steroid</td>
<td>Triyl methene</td>
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<tr>
<td>Color</td>
<td>Red-brownish</td>
<td>Dark Green</td>
<td>Dark Green</td>
<td>Dark blue</td>
<td>White</td>
<td>Blue</td>
<td>Dark blue</td>
<td>White</td>
<td>Blue</td>
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<td>Affinity to ILM</td>
<td>Low</td>
<td>High</td>
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<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Moderate</td>
<td>Unknown</td>
<td>High</td>
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<td>Affinity to ERM</td>
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<td>Low</td>
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<td>Low</td>
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<td>High</td>
<td>Moderate</td>
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<td>Toxicity to RPE</td>
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<td>Unknown</td>
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<td>Toxicity to neuroretina</td>
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<td>Moderate</td>
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<td>Unknown</td>
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</table>
solution of 248-275 m mol/kg. The iodine free IfCG is dissolved in 5 % glucose solvent generating an isoosmotic solution of 294 – 314 m mol/kg. IfCG in concentrations of 0.5 mg/ml selectively stains the ILM just like ICG and has a much safer profile for intravitreal use.

Trypan Blue

The anionic hydrophilic azo dye trypan blue has a chemical formula C$_{34}$H$_{24}$N$_{6}$O$_{14}$S$_{4}$ and a molecular weight of 960 Daltons.

This vital stain traverses the cell membrane in dead cells thereby staining the dead tissues blue. In the 1990s Trypan blue was first used intraocularly to stain the anterior lens capsule to facilitate capsulorrhesis for cataract surgery. The use of trypan blue in chromovitrectomy is limited to the staining of ERM. It stains the ILM minimally sometimes necessitating repeated application to facilitate visualization. Trypan blue staining of ERM helps mark out its entire extent thereby ensuring complete removal.

Clinical studies have clearly demonstrated that Trypan blue exerted little or no toxic effects on the retina. Experimental data, however disclosed evidence of retinal toxicity following trypan blue staining. Luke et al reported irreversible damage to the retina after exposure to trypan blue in a bovine model. In contrast to this report Jin et al, and Narayanan et al showed that damage to the rodent neurosensory cells was dose dependent and the toxicity could be eliminated at lower doses. A new indication for the use of trypan blue was to stain the edges of an open retinal tear on subretinal administration facilitating its identification during vitrectomy.

Triamcinolone Acetonide (TA)

Triamcinolone Acetonide (TA) is a synthetic insoluble corticosteroid, with a chemical formula C$_{24}$H$_{31}$FO$_{6}$ and a molecular weight of 434 daltons. The white steroid suspension has been used for chromovitrectomy since 2003 to visualize the transparent vitreous gel and the posterior vitreous cortex. Guo et al compared the effectiveness of four biostains (triamcinolone acetonide, indocyanine green, trypan blue and sodium fluoroscein) in delineating the vitreous and reported the best visibility and contrast following use of triamcinolone acetonide. In addition triamcinolone crystals get deposited on the ERM and ILM making their identification and peeling easier. The safety of triamcinolone acetonoide to the retina has been demonstrated by several invivo and in-vitro studies. Marison VL et al demonstrated that the vehicle (benzyl alcohol) can induce toxicity to the retina in rabbit models and hence the use of preservative free triamcinolone acetonide is recommended.

Patent Blue

Patent Blue is a hydrophilic anionic triylmethane dye with a chemical formula of C$_{27}$H$_{31}$N$_{2}$NaO$_{6}$S$_{2}$ and a molecular weight of 582 Daltons. This dye has been used to stain the anterior capsule during cataract surgery in a concentration of 0.24 % . Patent blue exhibit minimal systemic toxicity, carcinogenicity and
mutagenicity. Preliminary clinical data demonstrates a moderate affinity of patent blue to ERM and vitreous and a poor affinity to the ILM. Toxicity studies revealed conflicting reports on retinal toxicity of patent blue. Luke et al demonstrated that patent blue exhibited mild and reversible retinal toxicity, whereas Westermeier et al showed that RPE cells exposed in vitro to patent blue showed no toxicity. Analysis of available data indicate a safer profile for patent blue in comparison to trypan blue, however the exact safe dosage of patent blue for intravitreal injection remains unclear.

**Brilliant Blue G**

Brilliant blue G, also known as Coomassie or acid blue, is a blue biostain with a chemical formula $\text{C}_{47}\text{H}_{48}\text{N}_{3}\text{S}_{2}\text{O}_{7}\text{Na}$ and a molecular weight of 854 Daltons. Human and animal studies on the use of BBG for chromovitrectomy and anterior lens capsular staining were published in 2006. These study results indicate a safe clinical profile for both capsular staining and chromovitrectomy. Absence of corneal endothelial cell damage, no significant retinal pathological changes on light and electron microscopy, no reduction in ERG waves, and no clinical evidence of long term toxicity, were the hallmark results of these studies.

The remarkable affinity to the ILM and absence of toxicity makes it a first real alternative to ICG and IfCG.

**Bromophenol Blue (BrB)**

(Tetrabromophenolsulfonaphthalein) has a molecular weight of 670 Daltons and a chemical formula $\text{C}_{19}\text{H}_{10}\text{Br}_{4}\text{O}_{5}\text{S}$. In cataract surgery BrB represents an appropriate biostain at a concentration of 1.2 % to stain the anterior lens capsule intensively facilitating easy removal.

Preclinical experiments on six novel vital dyes for chromovitrectomy (Light green yellowish, E68, BrB, Chicagoblue, Rhodamine, Rhodulin blau- basic) showed that BrB stained the ERM and ILM better, and was free of toxicity at concentration of 1.2 % and 0.02 %. Similar reports have been obtained from histopathological studies. At a higher concentration of 1 % and 2 % enhanced ILM colouring was possible. Further human clinical data on its safety profile and defining its dosage and indications in chromovitrectomy is awaited.

**Sodium Fluorescein**

Sodium Fluorescein is a hydrophilic xanthene dye with a chemical formula $\text{C}_{20}\text{H}_{10}\text{Na}_{2}\text{O}_{5}$ and a molecular weight of 376 Daltons. Abrams And coworkers in 1978 demonstrated the efficacy of intravitreally injected sodium fluoroescin in staining the vitreous thereby aiding its complete removal. The toxicity of sodium fluoroescin to the retina has not been reported.

**Fluoromethalone Acetate (FMA)**

Fluoromethalone Acetate (FMA) is a synthetic fluorinated glucocorticosteroiud with an empirical formula $\text{C}_{24}\text{H}_{31}\text{FO}_{5}$ and a molecular weight of 418 Daltons. Hata et al performed pre-clinical investigations of fluromethalone acetate as a potential new adjuvant during vitreous surgery. They found neither reduction in ERG or histological changes following the use of Fluoromethalone acetate and concluded that FMA could be used as an alternative to TA during chromovitrectomy.

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<table>
<thead>
<tr>
<th>Sl.No</th>
<th>Technique</th>
<th>Procedure</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Air- filled Technique</td>
<td>FAE to remove fluid from vitreous cavity before dye injection.</td>
<td>Concentrates dye at posterior pole.</td>
<td>Higher retinal toxicity.</td>
</tr>
<tr>
<td>2</td>
<td>Fluid- filled technique</td>
<td>Inj of dye intravitreously into the BSS/ RL filled eye.</td>
<td>Immediate dye washout lesser retina toxicity.</td>
<td>Less staining as dye is washed out rapidly.</td>
</tr>
<tr>
<td>3</td>
<td>VINCE (Vitreoretinal Internal limiting membrane color enhancer.)</td>
<td>Painting brush constructed of a silicone tube connected to a 20 G metal cannula, Diluted dye in silicone cartridge.</td>
<td>Selective staining</td>
<td>Not patented.</td>
</tr>
</tbody>
</table>
Key issues

1. Chromovitrectomy improved the visualisation of preretinal structures in vitreoretinal surgery.
2. Intravitreal injection of dyes appear to be the most feasible approach to stain the vitreous and preretinal tissues.
3. Lower dye concentration and shorter exposure time can limit side effects.
4. Various techniques are available to stain ILM (Table 3)

Newer vital dyes exhibiting selective staining of tissues can limit side effects.

References


