Experimental Staining of the Anterior Lens Capsule in Albino Rabbits Using Capsulorhexis Marker

MOHAMED A. HASSABALLA, M.D., F.R.C.S.(Ed)
The Department of Ophthalmology, Faculty of Medicine, Cairo University and El-Rowad Eye Hospital

Abstract

Purpose: The aim of this study was to assess the toxicity of gentian violet, applied directly on the anterior capsule by the lower edge of capsulorhexis marker, to intraocular structures especially the corneal endothelium and retina.

Setting: Wet Lab. Kasr El-Aini Hospital.

Methods: This prospective comparative experimental study included 20 eyes of 10 albino rabbits that were assigned into two groups; group 1 (experimental, in which the gentian violet was used) and group 2 (control, without gentian violet). The right eye of each rabbit was put in group 1 and the left eye was put in group 2. The animals were divided into 2 subgroups; the first was killed 1 day after surgery and the second, after 1 week. The eye balls were enucleated and immediately fixed in bouin’s solution and embedded in paraffin. It was examined by light microscope.

Results: Light microscopy examination of multiple sections from each eye showed normal cornea iris tissue, ciliary body epithelium and lens epithelium. In one eye of the study group, there was loss of ganglion cells from neurosensory retina one day after surgery.

Conclusions: Staining the anterior capsule by gentian violet using the capsulorhexis marker is a relatively safe procedure with minimal histologic abnormalities. Further studies are needed using larger number of animals to exclude retinal toxicity.

Key Words: Capsulorhexis marker – Staining – Lens – Capsule – Albino – Rabbits.

Introduction

OPHTHALMIC surgical dyes have become valuable tools and are now widely used for both anterior and posterior segment indications. These dyes enhance contrast between the anterior capsule and the cortex during continuous curvilinear capsulorhexis (CCC) [1-3]. These dyes have also been found helpful for the following: To find the leading edge of a lost capsulorhexis [4], to visualize the anterior capsule tear in cases of traumatic cataract [5], to facilitate capsulorhexis during phacoemulsification of corneal opacities [6], to facilitate removal of posterior capsule plaque after cataract surgery or assist in posterior CCC in children [7], and to assist in training surgeons in phacoemulsification techniques [8].

Worldwide experience has demonstrated that capsular staining is safe and effective. Safety, however, depends on the specific dye used and its formulation. In 1993, Hoffer and McFarland [9] were the first to advocate using dye (fluorescein 2%) to stain the anterior capsule. In 1998, Fritz [10] demonstrated the use of fluorescein coupled with a blue filter; the only untoward effect was corneal edema in 2 eyes. Anterior capsule staining with gentian violet in humans was first presented in 1998 [11]. The study found no intraoperative or postoperative complications at a concentration of 0.01%. Horiguchi et al. [12], first published a report on the use of 0.5% indocyanine green (ICG) to stain the anterior capsule in 1998. In 1999, Melles et al. [13] published their use of 0.1% trypan blue dye to stain the anterior capsule in a series of 30 patients with mature cataracts. As with the aforementioned study by Horiguchi et al., there was no clinical evidence of increased inflammation, corneal endothelial impairment, or elevated IOP. Dada et al. [14] randomized 50 eyes to capsular staining with five different agents (10 eyes each): 0.1% trypan blue; 0.001% gentian violet; 0.5% ICG; 2% fluorescein; and autologous hemocoloration. The first three dyes provided superior visualization of the capsule and higher success rates in completing a capsulorhexis with no cases of extended capsular tears. The last two groups both had poorer visualization of the capsulorhexis and an extension rate of 20%.

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(mentioned in details in another study by the author under publication) to intraocular structures especially the corneal endothelium and retina.

**Material and Methods**

This prospective comparative experimental study included 20 eyes of 10 albino rabbits weighing between 2 and 4 kg that were assigned into two groups; group 1 (experimental, in which the gentian violet was used) and group 2 (control, without gentian violet). The right eye of each rabbit was put in group 1 and the left eye was put in group 2.

**Surgical technique:**

The basic surgical technique was as follows: The rabbits were anesthetized with an intramuscular injection of xylazine (5 to 8mg/kg) and ketamine hydrochloride (35 to 44mg/Kg). After the pupils were dilated with tropicamide and phenylephrine 10% eye drops, a 3mm limbal corneal tunnel was performed, and then viscoelastic material was injected into the anterior chamber. Afterwards, the wound was widened to 6mm to allow easy introduction of the instrument. In 5 eyes of the experimental group, the lower edge of the circular part of the capsulorhexis marker was stained by viscot surgical marker pen (gentian violet dye). In the other 5 eyes of the experimental group, the lower edge of the circular part of the capsulorhexis marker was stained by viscoat surgical marker pad (Vismark, gentian violet pad, Viscot medical, IIC). In the remaining 10 eyes (group 2) the capsulorhexis marker was not stained. In both groups, the capsulorhexis marker was introduced into the anterior chamber and applied to the anterior capsule for few seconds without pressure to avoid zonular stress. Closure of the wound was done by 2 10/0 nylon sutures. All animals were kept in the animal house and were handled according to the Association for Research in Vision and Ophthalmology Statement for Use of Animals in Ophthalmic and Vision Research. The animals were divided into 2 subgroups; the first was killed 1 day after surgery and the second, after 1 week. The eye balls were enucleated and immediately fixed in bouin’s solution, dehydrated in ascending grades of alcohol (70, 90, 100%) cleared in xylol and embedded in paraffin. Then, the embedded specimens were sectioned (5-7mm thickness) and stained with Hematoxylin and eosin stains. It was examined by light microscope.

![Fig. (1): A photomicrograph of cornea of a control group rabbit showing the flat endothelial cells with their flat nuclei (thick arrow) resting on Descemet’s membrane (thin arrow). H & E x400.](image1)

![Fig. (2): A photomicrograph of cornea of an experimental group rabbit showing normally appearing flat endothelial cells (thick arrow) and intact Descemet’s membrane (thin arrow). H & E x400.](image2)

![Fig. (3): A photomicrograph of a section in a control rabbit retina showing photoreceptors layer (1), outer nuclear layer (2), outer reticular layer (3), inner nuclear layer (4), inner reticular layer (5) and ganglion cell layer (6). H & E x400.](image3)

![Fig. (4): A photomicrograph of a section in an experimental rabbit retina showing a ganglion cell with pyknotic (dark) nucleus (thick arrow) and another one (thin arrow) that is displaced within the inner reticular layer (IRL). H & E x400.](image4)
Results

All the surgeries were uneventful. In the first postoperative day, 3 rabbit eyes had moderate to severe inflammatory reactions with cells and flare in the anterior chamber and corneal edema (2 eyes in the experimental group and 1 eye in the control group). In all these eyes, the inflammatory reaction and corneal edema resolved completely. The other rabbit eyes presented a mild inflammatory reaction in the first 2 days that resolved completely.

Histopathologically, no eye in the study or control group showed any sign of corneal toxicity (Figs. 1,2). Light microscopy examination of multiple sections from each eye showed normal iris tissue, ciliary body epithelium and lens epithelium. The retina was normal in all eyes of the control group (Fig. 3) and the experimental group except one eye of the experimental group, there was loss of ganglion cells from neurosensory retina one day after surgery (Fig. 4).

Discussion

The development of the CCC has contributed significantly to the safety and effectiveness of cataract extraction and IOL implantation [15]. This technique produces a strong rim that resists tearing even when stretched during crystalline lens removal or IOL implantation [16]. Staining the anterior capsule with dye is by far one of the most important advances in the management of cataract. Dada et al. [14] reported that trypan blue, ICG, and gentian violet are equally effective in staining the anterior capsule in eyes with white cataract without producing toxic effects.

Gentian violet (hexamethyl rosaniline chloride) is a triphenylmethane (rosaniline) dye. The pure compound is known as crystal violet. It was chosen in this study because it is cheap, easily available, intensely and homogeneously stains the anterior capsule. Moreover, Gentian violet is bacteriostatic and bactericidal to gram-positive bacteria and to many fungi and thus may help in prevention of postoperative endophthalmitis [17].

Different techniques has been proposed for staining of the anterior capsule by different dyes. Many surgeons continue to inject capsular dye beneath an air bubble, as originally described by Horiguchi et al. [12] and Melles et al. [13]. The ophthalmologist fills the anterior chamber with air using a 30-gauge cannula inserted through a tiny paracentesis. The surgeon then draws several drops of dye into a tuberculin syringe and places them on the anterior capsular surface by means of the same cannula. The bubble prevents excessive dilution and helps to confine the dye to the capsular surface. After waiting at least 10 to 15 seconds, the surgeon can easily irrigate out the dye with BSS before injecting-viscoelastic.

Laureano and Coroneo [18] suggested simply omitting the air bubble. Kayikciglu et al. [19] have used a viscoelastic-mixing technique with 0.4% trypan blue. In this study the dye was applied directly onto the anterior surface of the capsule by using the capsulorhexis marker and this help to decrease the amount of the dye inserted in the anterior chamber and also avoids inadvertent application of the dye towards the corneal endothelium.

Many studies investigated toxicity of Gentian violet. Previously, gentian violet 0.1 % with methylene blue 1% was used for staining [13]. This combination is no longer used because the endothelial toxicity of the dyes causes corneal edema. Ünlü et al. [17] examined the effects of gentian violet 0.001% and gentian violet 0.01% without methylene blue and found that each concentration of gentian violet alone was effective in staining the anterior capsules of white cataracts and found that both concentrations had no toxic effects on intraocular tissues. Gamal Eldin and coauthors20 reported that gentian violet 0.05% to 2% stained the anterior capsule of the rabbit eye effectively but that only the lowest concentration (0.05%) showed excellent preservation of the cornea during 1 week of observation.

In this study, no toxic changes were evident in the cornea. Only one eye had partial ganglion cell loss from the retina. This is most probably related to preparation problems rather than actual toxicity because it occurred in the first post operative day and no toxic changes were evident in all other eyes examined in the first postoperative day and those after one week.

The rabbit model was chosen because of the gross morphologic similarities between rabbit and human corneas, lower cost, and the general familiarity of the medical community with the rabbit model [20]. However, rabbit corneal endothelium may regenerate after injury [21] while human corneal endothelium recovers by the growth in size and migration of endothelial cells [22]. To study corneal endothelial toxicity, cat and monkey eyes are better models [21].

In conclusion, staining the anterior capsule by gentian violet using the capsulorhexis marker is a
relatively safe procedure with minimal histologic abnormalities. Further studies are needed using larger number of animals to exclude retinal toxicity.

Acknowledgment: I would like to express my deep gratitude to Dr. Dina Helmy (Associate Professor of Histology, Cairo University) for her help and support in preparing and interpreting the slides.

References