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Does Precipitation Reduce Tissue Staining by Indocyanine Green Dye Solutions?

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Purpose: Indocyanine green (ICG) dye precipitates when mixed with certain ophthalmic irrigation solutions. The purpose of this study is to investigate whether this precipitation reduces ICG staining of the anterior lens capsule.

Methods: ICG was diluted with each of the following solutions: BSS Plus, physiological saline, or Opeguard Neo. The products were then examined for green precipitate by light microscopy. The tissue staining capability of each ICG solution was tested at two different concentrations (0.5% and 0.0625%) in porcine lenses, regardless of whether the solution contained precipitate.

Results: Green precipitate was observed in both concentrations of ICG solutions diluted with BSS Plus, but not in the solutions diluted with either physiological saline or Opeguard Neo. As assessed with light microscopy, staining of the anterior lens capsule appeared weaker for all 0.0625% ICG solutions compared to the corresponding 0.5% ICG solutions. The precipitate that formed in the 0.5% ICG solution diluted with BSS Plus had little effect on the staining quality of the anterior lens capsule. In contrast, the 0.0625% ICG solution diluted with BSS Plus (w/precipitate) showed weaker staining in the lens capsule compared to the other two 0.0625% ICG solutions (w/o precipitate).

Conclusion: These results suggest that precipitation of ICG may weaken its capability to stain the anterior lens capsule or other transparent ocular tissues. Therefore, ICG solutions that do not form a precipitate may be more capable of staining tissues at lower concentrations. As for other possibilities to explain the deterioration in staining, the effect of the composition of BSS Plus should also be considered. **Jpn J Ophthalmol 2003;47:18–21** © 2003 Japanese Ophthalmological Society

Key Words: Anterior capsule, indocyanine green dye, internal limiting membrane, precipitation, tissue staining.

Introduction

Indocyanine green (ICG) dye has been used in ophthalmic surgery to facilitate the visualization of transparent ocular tissues such as the anterior lens capsule[1] and the retinal internal limiting membrane.[2] Recently, we further demonstrated its usefulness in the visualization of conjunctival cysts for complete cyst removal.[3] Usually, we dissolve 25 mg of ICG powder in 1 mL of sterilized distilled water and then dilute this solution five times by adding 4 mL of commercially available ophthalmic irrigation solution (BSS Plus; Alcon, Fort Worth, TX, USA; see Table 1 for detailed composition) for staining of either the anterior lens capsule or the retinal internal limiting membrane. However, with this mode of preparation, we sometimes notice small green particles in the prepared ICG solution, which could theoretically influence the staining capability of ICG. In the current study, we investigated whether this type of precipitate formation reduces the capability of ICG to stain the anterior lens capsule of porcine eyes.

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Table 1. Detailed Composition of BSS Plus Formulation

Composition	Concentration (mM)
Sodium chloride	122.2
Potassium chloride	5.08
Calcium chloride dihydrate	1.05
Magnesium chloride hexahydrate	0.98
Dibasic sodium phosphate	3.00
Sodium bicarbonate	25.00
Dextrose	5.11
Oxidized glutathione	0.30
pH	7.4
Osmolality (mOsm/kg)	305

Materials and Methods

Experiment 1

We first dissolved 25 mg of ICG (Diagnogreen; Daiichi Pharmaceutical, Tokyo) in 1 mL of distilled water (hereafter referred to as conc. ICG solution), and then the conc. ICG solution was further diluted 5 times by adding 4 mL of one of the following solutions: BSS Plus, physiological saline (154 mM NaCl), or another kind of commercially available ophthalmic irrigation solution, Opeguard Neo (Opeguard Neo Kit; Senju, Osaka; see Table 2 for detailed composition). The products were then examined for green precipitate by light microscopy. The experiment was performed at room temperature (24°C).

Experiment 2

The tissue-staining capability of each type of ICG solution was tested in porcine lenses at two concentrations (0.5% or 0.0625% ICG). To make 0.5% or 0.0625% ICG solutions, the conc. ICG solution was diluted 5- or 40-fold by adding 4 mL or 39 mL, respectively, of one of the following solutions: BSS Plus, physiological saline, or Opeguard Neo. Ten minutes after dilution, porcine crystalline lenses were immersed for 30 seconds in each of the ICG solutions, as prepared above, and subsequently rinsed with 100 mL of physiological saline. The lens surfaces were first inspected with an operating microscope, and then pieces of the anterior lens capsules were resected and subjected to light microscopy. To ensure the repeatability of the results, 2 eyes were used in each experiment. The experiment was performed at room temperature (24°C).

Table 2. Composition of Opeguard Neo® Formulation

Composition	Concentration (mM)
NA^+	153.10
K^+	5.08
Ca^{2+}	1.04
Mg^{2+}	0.98
HCO_3^-	25.04
CI^-	122.58
CH_3COO^-	4.40
Citrate ion	3.40
Dextrose	5.10
Oxidized glutathione	0.30
pH	7.6
Osmolality (mOsm/kg)	310

Results

Experiment 1

Green precipitate was first observed approximately 2 minutes after the conc. ICG solution was diluted with BSS Plus, and continued to increase up to 3 minutes after dilution. In contrast, green precipitate was never observed after dilution of ICG with either physiological saline or Opeguard Neo, even after 2 hours.

Experiment 2

The staining of the anterior lens capsule appeared weaker when stained with any of the 0.0625% ICG solutions compared to the correlating 0.5% solutions, regardless of the diluent. Green precipitate was noted in both concentrations of ICG solutions diluted with BSS Plus. In contrast, no precipitate was observed at either ICG concentration when diluted with the other two solutions. Light microscopy revealed that the precipitate had little effect on the staining capability of the 0.5% ICG solution diluted in BSS Plus in anterior lens capsules. However, inspection with both operating and light microscopes revealed that the 0.0625% ICG solution diluted with BSS Plus (with precipitate) showed weaker staining compared to the other two 0.0625% ICG solutions (without precipitate) (Figures 1A, 1B). The degree of staining was consistent in the 2 eyes tested each time. By light microscopy, the staining of the anterior lens capsule might have been weaker when stained with Opeguard Neo compared to physiological saline (Figures 1B, h, i). However, the difference was indistinguishable under the operating microscope (Figures 1B, e, f).

Discussion

Generally speaking, intraoperative use of adjunctive agents that do not have a proven intraocular

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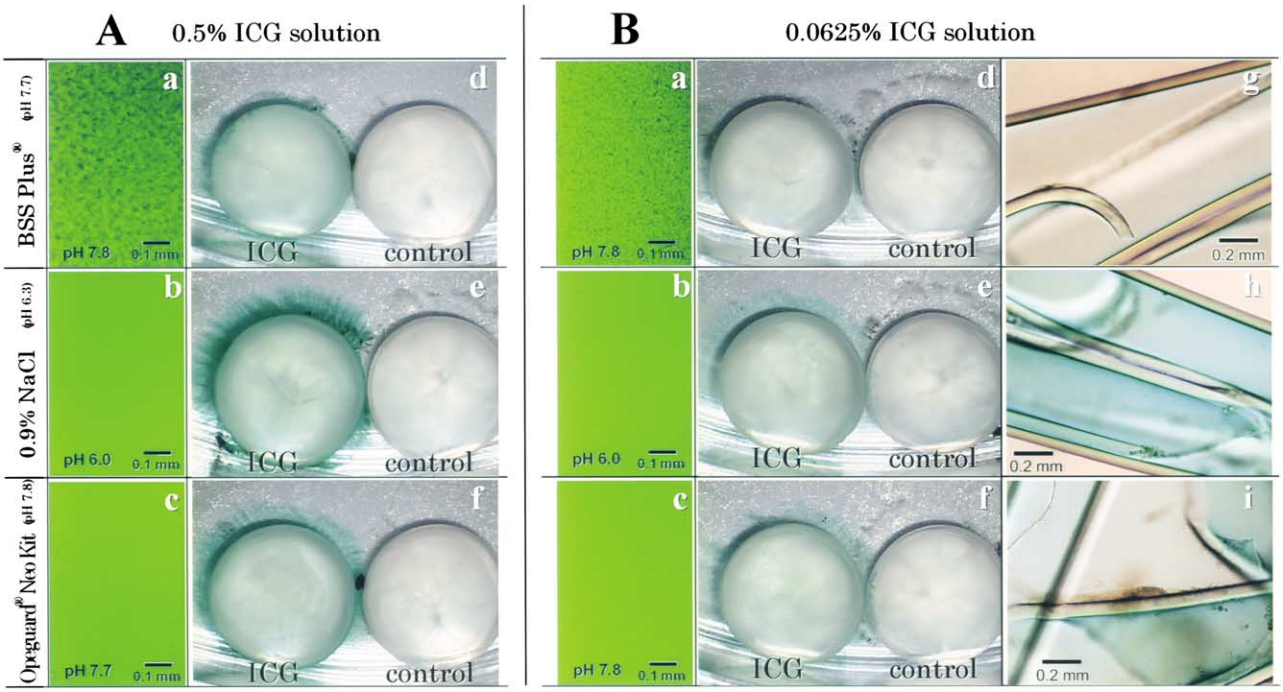

Figure 1. (**A**) Results of experiment 2 using 0.5% indocyanine green (ICG) solutions. Green precipitate was noted only when ICG was diluted with BSS Plus solution (**a**–**c**). However, precipitate formation had little effect on the staining quality of ICG in the anterior lens capsule (**d**–**f**). (**B**) Results of experiment 2 using 0.0625% ICG solutions. For all diluents, staining of the anterior lens capsule appeared weaker with the 0.0625% ICG solution compared to the corresponding 0.5% ICG solution (**d**–**f**). Green precipitate was noted only in the 0.0625% ICG solution diluted with BSS Plus (**a**–**c**). Examination with light microscopy of the stained anterior capsule revealed that the BSS Plus solution yielded weaker staining compared to the other two 0.0625% ICG solutions. Resected anterior capsule was partly rolled (**g**–**i**).

safety profile should be minimized. Although ICG staining of the anterior lens capsule and the retinal internal limiting membrane has greatly facilitated cataract surgery and macular surgery, respectively, ICG is potentially hazardous. For example, we experienced a case of hypermature cataract with anterior lens displacement in which ICG injected into the anterior chamber to stain the anterior lens capsule migrated into the posterior segment and stained the retina. Recently, Enaida et al revealed that the intravitreous injection of 0.025 mg/mL (0.05 mL/eye) induced functional damage of the rat retina without any apparent morphological damage.[4] Therefore, ICG concentrations should be kept to a minimum, even for anterior segment surgery. The results of experiment 2 suggested that the ICG solutions that do not form precipitate (physiological saline or Opeguard Neo) may be more capable of staining tissues at lower concentrations compared to the ICG solutions that form precipitate (BSS Plus). As for other possibilities to explain the deterioration in staining, the effect of the composition of BSS Plus should also be considered.

In addition, ICG precipitates could potentially get entrapped in the vitreous, so ICG precipitation must be avoided. Because Opeguard Neo did not induce precipitation under the present protocol, diluting the conc. ICG solution with Opeguard Neo would be reasonable for intraoperative use. Alternatively, when Opeguard Neo is not available, physiological saline may be sufficient. We showed that the ICG solution (pH 6.0) diluted with saline had no precipitate even after 2 hours. However, injection of a solution with a pH of 6.0 may be a problem in clinical use.

The mechanism for ICG precipitation is currently not known. Our preliminary experiments have revealed that ICG precipitation occurs in various salt solutions, including simple hypertonic saline (231 mM), but not in hyperosmotic glucose solution (462 mM), which suggests that this is an ion-related but not an osmolarity-related phenomenon (data not shown). Further study is needed to elucidate the mechanism of ICG precipitation. Also, as suggested by the result of light microscopic observation in experiment 2, the staining capability of Opeguard Neo might be weaker compared to physiological saline, indicating the pos-

sibility of the influence of the ingredients of the Opeguard Neo formulation.

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