Original Article

Observation of Persistent Fundus Fluorescence after Internal Limiting Membrane Peeling Assisted by Indocyanine Green Solution of Different Concentrations

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Abstract

Purpose: To investigate how long indocyanine green (ICG) remains in the fundus after vitreoretinal surgery assisted with ICG, and to identify factors that influence the persistence duration.

Methods: Fifty five eyes diagnosed as idiopathic macular hole (Stage 2 and 3) were randomly divided into five groups. ICG solution at concentrations of 5, 2.5, 2.5, 1.25, and 0.5 mg/ml, employed in cases of Group I to V respectively, was applied to stain the internal limiting membrane (ILM) during the procedure of internal limiting membrane peeling. A prospective study was carried out after pars plana vitrectomy and ILM peeling were performed on 55 eyes with Stage 2, 3, or 4 idiopathic macular holes. Infrared fundus pictures were obtained in all patients before and after surgery.

Results: High levels of fluorescence from residual ICG (ICG hyperfluorescence) were mainly localized at the posterior pole of the fundus after surgery. In Group Ⅰ, Ⅱ, Ⅲ, Ⅳ and Ⅴ, the duration of persistence of flurorescence from ICG was 8.33±0.87, 3.59±0.94, 3.75±0.79, 2.30±0.48, and 1.29±0.49 months, respectively. Although no significant difference was detected between Group Ⅱ and Group Ⅲ, the general inter-group difference was significant among the five groups in which different ICG concentration was applied. In Group Ⅲ, even though 90% of the macular holes acquired anatomical closure, ICG hyperfluorescence was detected in the macular area.

Conclusion: ICG remains in the fundus for a period of months. The persistence duration of fluorescence from ICG is positively correlated with the concentration and the staining time of ICG. Hyaluronan is beneficial in reducing the amount of ICG residue in the macular area.

Keywords: macular hole, internal limiting membrane, indocyanine green

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Introduction

A standard procedure for the surgical treatment of macular hole is to peel the internal limiting membrane (ILM). However, the ILM is difficult to visualize because of its thin and transparent nature. Indocyanine green (ICG) has been shown to facilitate ILM peeling by improving the visualization of ILM. However, ICG is known to remain in the fundus of the eye for a period of time after surgery. We designed this study to determine the duration of ICG persistence and identify factors that may influence ICG persistence after ICG-assisted peeling of the ILM.

Patients and Methods

Fifty-five consecutive eyes of 55 patients diagnosed as idiopathic macular hole (MH) were classified into four stages according to the Gass Classification System[1], and randomly divided into five groups (Table1). All subjects in this study were treated at Zhongshan Ophthalmic Center.

All patients underwent a complete set of ophthalmic examinations before surgery, which included measurement of the best corrected visual acuity (BCVA), slit-lamp biomicroscopy combined with indirect funduscopy, and optical coherence tomography (OCT). Zeiss retina angiography (type: FF450plus

IR) was performed to obtain ICG fluorescence and autofluorescence images. We adjusted the incident wavelength to 805 nm for infrared reflectance imaging and, used 28 illuminations to properly observe the ICG fluorescence.

The ICG solutions were prepared as follows: 25 mg of sterile ICG powder was reconstituted with 1.0 ml of sterile water for injection. After the crystalline ICG was completely dissolved, the solution was diluted in balanced salt solution (BBS Plus; Alcon Laboratories, Inc.) to generate ICG solutions at the concentrations of 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml and 0.5 mg/ml. Table 1 was not included in this document, not sure if the information that Group Ⅱ and Ⅲ shared the same concentration was included in Table 1.

Standard pars plana vitrectomy was performed on each eye. Fluid-air exchange was performed after the posterior vitreous was separated. Hyaluronan was used to temporarily cover the macular hole in Groups Ⅰ and Ⅱ. We did not use anything to cover the macular holes in Groups Ⅲ, Ⅳ, and Ⅴ. Then, 0.2 ml of ICG solution was gently injected into the posterior vitreous cavity, kept in the vitreous cavity for no more than five seconds and removed by aspiration. When the ILM was properly stained, fluid-air exchange was performed again. The hyaluronan was removed by vitreous cutter. Then, the ILM was peeled using intraocular forceps as continuously as possible. A circular area of about 4 optical disk di ameter was removed. Fluid-air exchange was performed, which was followed by air-gas exchange using 14% or 16% perfluoropropane. All procedures in our study were performed by a single surgeon using standardized techniques. Patients were asked to maintain a face down position and use antibiotic and steroid eye drops for two weeks after surgery.

Patients were followed on monthly basis after surgery until the ICG fluorescence disappear. The follow-up examinations included BCVA, OCT and infrared reflectance fundus photography and indirect funduscopy under slit-lamp biomicroscopy.

The statistical analysis of the ICG persistence duration was performed using SPSS13.0 software. The Mann-Whitney U-test was used to analyze the ICG residual time in two independent samples among the groups (Table 2). The data (including age, sex, and stage of macular hole) were analyzed by K-W-test for multiple-independentsamples. $P<0.05$ was considered significant.

Results

The persistent duration of residual ICG fluorescence in each group is listed in Table 1. No autofluorescence was observed before surgery by photographing infrared reflectance of the fundus. The persistent duration of ICG fluorescence was significantly different among the groups in which different concentrations of ICG solution were applied.

We also found that ICG hyperfluorescence was mainly detected at the optic disc, the nerve fiber layer around the optic disc, and the macular hole bed, the area where the macular hole existed before the operation (Figures 1~5). No ICG hyperfluorescence was found in the macular hole bed in Groups Ⅰ, Ⅱ, Ⅳ, and Ⅴ shortly after surgery. However, in Group Ⅲ, ICG hyperfluorescence was detected in the macular hole bed even if the anatomical closure rate of the macular hole reached 90% (Figure 3).

Discussion

Two factors influenced the residual amounts of ICG after surgery, the concentration and staining time. In our study, the longest persistence duration of residual ICG was in Group Ⅰ, in which the highest concentration of ICG was applied. The persistence duration differed significantly when different concentrations of ICG were used. However, there was no significant difference in persistence duration of fluorescence from residual ICG between Group Ⅱ and Group Ⅲ, in which the same concentration of ICG was used. We concluded that the persistence duration of fluorescence from residual ICG was positively correlated with the concentration of ICG used during the operation.

Our results differed from those of some other previous studies, possibly because of differences in the staining time. For example, using an ICG concentration of 5 mg/ml for 1 min, Machida et al[2], observed 12 cases and found that hyperfluorescence of ICG remained even at 12 months after surgery in all cases. This was obviously longer than the persistence

duration observed in Group Ⅰ (8.33±0.87 months) of the current study, in which the same concentration of ICG was used. Tadayoni et al[3] reported that using 2.5 mg/ml ICG for 3 min can cause fluorescence in the optic disc area after macular hole surgery to persist for 6~7 months. However, in our study, the persistence duration of fluorescence from residual ICG in Groups Ⅱ and Ⅲ, which also were treated with an ICG concentration of 2.5 mg/ml, were 3.59±0.94 months and 3.75±0.79 months, respectively.We assume that the relatively shorter persistence duration of fluorescence in our series was due to the short staining time. Horiguchi et al[4] peeled ILM using 1.25 mg/ml ICG staining for 10 to 30 seconds, which was similar to the procedure applied in Group Ⅳ of our study. The persistence time of hyperfluorescence was also similar (2.7±1.4 months in Horiguchi's study vs. 2.30±0.48 months in ours). Thus, our results suggest that the persistence duration of fluorescence from residual ICG was positively correlated with the staining time.

The location of ICG hyperfluorescence after surgery

In the current study, we found that ICG hyperfluorescence was mainly detected at the optic disc, the nerve fiber layer around the optic disc, and the macular hole bed, which was similar to the observations by other authors[2,4,5]. Furthermore, the fluorescence clearly remained longer at the optic disc than in the nerve fiber layer around the optic disc and the macular hole bed in our study. Weinberger et al[6]. concluded that the persistent signal was due to slow metabolism of the ICG in these places. Horiguchi, et al[4]. concluded that, because the ILM was thinnest at the optic disc, ICG might pass more easily through the ILM into the nerve tissues and remain in the disc region for a longer duration. Machida, et al[2]. proposed at least two possible explanations to account for hyperfluorescence of the optic nerve head.The first, an anatomical explanation, relies on the fact that the optic nerve head is covered by the inner limiting membrane of Elschnig that is continuous with the ILM.The Elschnig membrane is thickened in the central part of the disk. ICG is known to stain the extracellular matrix including collagen, which is a constituent of the lamina cribrosa. Thus, the lamina cribrosa could be stained by ICG if intraocular administration of ICG diffuses deeply into the optic nerve head. Second, the optic nerve head may reflect fluorescence produced by the surrounding structures in the ocular fundus, which enhancing its own hyperfluorescence. Tadayoni et al[3] suggested that the ICG may bind to some components of ganglion cell axons and migrate into the optic nerve with the anterograde axonal transport. Paques et al[7] found that ICG undergoes fast bidirectional transport, which resulted in long existence of ICG fluorescence. During surgery, we found that ICG can permeate through the ILM.Therefore, it is possible that ICG passes through the ILM and stains the collagen of the lamina cribrosa, causing the long persistence duration.

We and other authors all observed an interesting finding that the area of the macular hyperfluorescence nearly corresponded to the previous macular hole area before the operation, even when the macular hole achieved complete closure. Machida et al[2] suggested that the macular hole may be a path by which ICG reaches the subretinal tissue and is trapped beneath the retina. Horiguchi et al[4] thought it was possible that ICG penetrated beneath the retina and/or diffused into the retina through the macular hole during surgery and remained for a longer duration in the foveal region after the operation.Several reports have suggested that ICG can be taken up by retinal pigment epithelium (RPE) cells through a saturable, cumulative and carrier-mediated transport mechanism. Also, the uptake of ICG by RPE showed an increase in the intracellular ICG concentration with any increase in the extracellular dye concentration[8-9]. In our study, ICG hyperfluorescence in the macular hole bed was only detected in Group III. We suggest that the hyaluronan prevented the ICG from contacting with the RPE cells and being absorbed at the base of the macular hole in Group Ⅰ and Ⅱ. However, in the groups Ⅳ and Ⅴ, no fluorescence was found shortly after surgery even though nothing was used to cover the macular hole in these groups before ICG staining. This result may have been due to the low concentration of ICG used. In these two groups, we demonstrated that ICG mainly persists at the optic disc and the nerve fiber around the optic

disc, with little of the ICG remaining in the macular area. The RPE cells at the base of the macular hole absorbed only a little ICG.Therefore, in the lower concentration groups, no fluorescence was found at the macular hole bed.

In summary, we conclude that ICG remains in the fundus for up to months after ILM peeling assisted with ICG stain. The persistence duration of fluorescence from ICG is positively correlated with the concentration and the staining time of the ICG. Hyaluronan used to cover the macular hole before staining is beneficial in reducing the residue of ICG in the macular area.

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