

Kazuaki Kadonosono  
Satoshi Takeuchi  
Kazuro Yabuki  
Tadashi Yamakawa  
Atushi Mekada  
Eiichi Uchio

## Absorption of short wavelengths of endoillumination in indocyanine green solution: implications for internal limiting membrane removal

Received: 27 August 2002  
Revised: 7 January 2003  
Accepted: 28 January 2003  
Published online: 15 March 2003  
© Springer-Verlag 2003

K. Kadonosono (✉) · S. Takeuchi  
K. Yabuki · E. Uchio  
Department of Ophthalmology,  
Yokohama City University Medical Center,  
4-57 Urafune-cho, Minami-ku,  
232-0024 Yokohama, Japan  
e-mail: kado@med.yokohama-cu.ac.jp  
Tel.: +81-45-253-5372  
Fax: +81-45-253-8490

T. Yamakawa  
Department of Endocrinology and Diabetes,  
Yokohama City University Medical Center,  
Yokohama, Japan

A. Mekada  
Department of Ophthalmology,  
Shiga University of Medical Science,  
Shiga, Japan

**Abstract Purpose:** To measure the absorption coefficients of short wavelengths, irradiated by a light source used for vitreous surgery, in indocyanine green (ICG) solution and balanced salt solution (BSS), and to determine the implications of these absorption coefficients on retinal phototoxicity caused by ICG-assisted internal limiting membrane removal. **Methods:** The absorption coefficients of short wavelengths irradiated by a commercially available light source used for vitreous surgery were measured in ICG solution and BSS using a dual-beam spectroradiometer. **Results:** The absorption coefficient of wavelengths irradiated by the endoillumination light source in ICG solution was almost the same as that obtained in BSS. The absor-

bance coefficients of the ICG solutions were  $0.18 \text{ cm}^{-1}$  at 400 nm and  $0.03 \text{ cm}^{-1}$  at 450 nm. In BSS, the absorption coefficients were  $0.17 \text{ cm}^{-1}$  at 400 nm and  $0.015 \text{ cm}^{-1}$  at 450 nm. No significant difference in absorbance was seen between 400 nm and 450 nm ( $P>0.05$ ). **Conclusions:** The absorption of wavelengths is not greater in ICG solution than in BSS. ICG solution during intravitreal use probably does not enhance retinal photochemical injury during ICG-assisted internal limiting membrane removal.

### Introduction

Indocyanine green (ICG) solution distinctly stains the invisible retinal internal limiting membrane (ILM) of the human eye [5, 8]. ICG staining greatly facilitates the removal of the ILM during macular hole surgery, leading to a higher hole closure success rate and better visual acuity [1, 9].

Some investigators, however, have recently reported that the intravitreal use of ICG solution might enhance phototoxicity when used in combination with endoillumination, resulting in retinal or retinal pigment epithelial atrophy [3, 11]. The short-wavelength light emitted by endoillumination during vitrectomy is a potential source of photochemical injury; several cases of endoillumination-related retinal phototoxicity occurring during epiret-

inal membrane peeling have been reported [10]. Photochemical damage is produced by short wavelengths of visible light [6], and histological changes are observed in retinal pigment epithelium and photoreceptor cells [7]. Commercially available light sources for endoillumination during vitrectomy emit a wide range of light wavelengths from short (approximately 400 nm) to long (approximately 700 nm) [13], while indocyanine green exhibits strong absorption in the near-infrared part of the visible spectrum (approximately 805 nm in human plasma). Therefore, the absorbance of short wavelengths of light in ICG solutions is an important subject requiring investigation.

If indocyanine green solution exhibits a higher absorbance of short wavelengths between 400 and 500 nm than balanced salt solution (BSS), it may have an en-

hanced photochemical effect on the underlying sensory retina when it is applied to the ILM or retinal pigment epithelium during a vitrectomy. However, the accurate transmission characteristics of short wavelengths of light, emitted during endoillumination, in ICG solution or BSS remain unknown [4].

In this study, we quantified the absorption coefficients of short wavelengths in ICG solutions and assessed whether ICG solutions would absorb the blue light that is hazardous to photoreceptors and the retinal pigment epithelium.

## Material and methods

The spectral distribution of light sources in ICG solution and BSS was determined using a standard fiberoptic endoillumination probe commonly used for vitreous surgery (Fiberoptic Endoillumination; Alcon, Dallas, TX, USA). ICG solution (0.1%, Daiichi, Tokyo, Japan) was prepared by diluting ICG in BSS.

Solutions were held in a vessel with a fused silica window at the base to maximize transmission. Above the vessel, a fused silica beam splitter was used to allow the simultaneous measurement of the initial and transmitted spectral radiation. The splitting ratio of the beam splitter was measured at each wavelength to allow the spectral radiation to be calculated.

The spectral distribution was measured with a resolution of 1 nm between 220 nm and 800 nm with a calibrated spectroradiometer (USR 40 V; Ushio Electronics, Tokyo, Japan). The measurements were repeated three times and performed at the power level recommended by the manufacturer. The output signal from the spectroradiometer was digitized and averaged by a personal computer with a digital board. The total absorbance of the sample and the sample holder in the sample chamber was measured, and the absorption coefficient of the ICG solution and the constituent components of BSS were measured by subtracting the absorbance of the sample holder from the total absorbance.

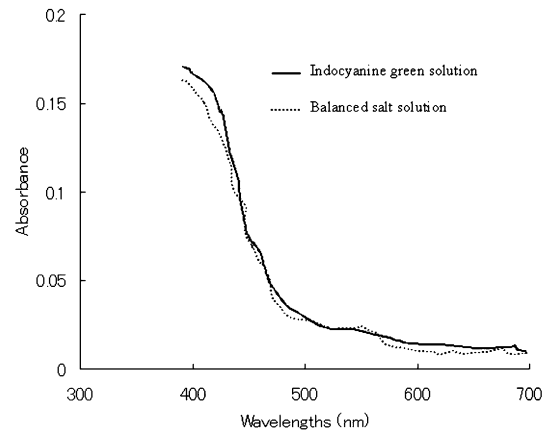
The Beer–Lambert law was used to determine the transmittance of each species contained in BSS as follows:  $T=II_0=(1-R)^2(1-R')^2e^{-\alpha mCd}$ , where  $I_0$  and  $I$  are the light irradiation at the front and rear surfaces of the sample holder;  $R$  and  $R'$  are the reflectivity of the glass–ICG solution and the glass–air interfaces, respectively;  $\alpha m$  is the molar absorption coefficient in moles per liter;  $C$  is the concentration in moles per liter; and  $d$  is the depth of the solution. The absorption coefficients ( $A$ ) in the ICG solution and BSS were measured using the dual-beam configuration of the spectrophotometer and obtained by subtracting the absorption of the sample holder ( $T'$ ) from the total absorbance ( $T$ ):

$$A = \log(T'/T) = 0.434\alpha mCd - 2\log(I - R) \cong 0.434\alpha mCd \quad (1)$$

In this manner, the molar absorbance coefficients in the constituent components of BSS were calculated using Eq. 1 or the short wavelengths irradiated by the light source. Finally, by summing the absorption coefficients at 400 nm and 450 nm from each species found in BSS, a total absorption coefficient for BSS at short wavelengths was obtained.

## Results

Absorbance levels in the ICG solution were almost equal to those in BSS for all the spectra measured (Fig. 1). The absorption coefficients in the ICG solution were deter-



**Fig. 1** Dependence of absorption in indocyanine green solutions and balanced salt solutions for short (approximately 400 nm) to long (approximately 700 nm) wavelengths

**Table 1** Molar absorption coefficient ( $\alpha m$ ,  $M^{-1}cm^{-1}$ )

Species	Molarity in BSS (mol/ml)	400 nm	450 nm
Sodium chloride	109.5	12.1	0.0007
Potassium chloride	10.0	8.1	0.0015
Calcium chloride	3.3	2.3	0.0008
Magnesium chloride	1.50	4.2	0.00034
Sodium acetate	28.7	2.3	0.34
Sodium citrate	5.71	3.23	0.39

**Table 2** Absorption coefficient ( $cm^{-1}$ ). BSS Balanced salt solution, ICG 0.1% indocyanine green solution

Solution	400 nm	450 nm
BSS	0.18	0.03
ICG	0.17*	0.015**

\*  $P=0.76$ , \*\*  $P=0.65$

mined to be  $0.18\text{ cm}^{-1}$  at 400 nm and  $0.03\text{ cm}^{-1}$  at 450 nm. Using Eq. 1, the molar absorptions for each species at wavelength of 400 and 450 nm were calculated. The results obtained for each species in BSS are shown in Table 1. On the basis of these results, the absorption coefficients in BSS were obtained by summing the varying molarity of solutions, which were determined to be  $0.17\text{ cm}^{-1}$  at 400 nm and  $0.015\text{ cm}^{-1}$  at 450 nm. No significant differences in absorption coefficients at 400 nm and 450 nm were observed between BSS and ICG (Table 2,  $P>0.05$ ).

## Discussion

The complete spectrum of short wavelengths irradiated by endoillumination light sources was examined in ICG

solution and BSS. The results showed that the absorption coefficient of short wavelengths of light in ICG solution was almost the same as that obtained in BSS. A study by Dair et al. investigated the absorption coefficients of BSS for ultraviolet (193 nm and 213 nm) wavelengths and reported them to be  $140 \text{ cm}^{-1}$  and  $6.9 \text{ cm}^{-1}$ , respectively [2]. Although the absorption of ultraviolet wavelengths in BSS has been reported, no reports have described the absorption of short wavelengths in BSS. The absorption coefficients of short wavelengths may be less than those of ultraviolet wavelengths, since the longer wavelengths have lower absorption levels. In this regard, the previous studies may support our results for the absorption rate of short wavelengths.

Recently, ILM staining using ICG has been reported to result in better visibility and the easier removal of the ILM during macular hole surgery [1, 9]. This surgical technique is a very useful tool for the surgical management of macular hole, epiretinal membrane and macular edema. The possibility of retinal phototoxicity as a result of ICG injections during vitrectomies is, however, of great concern to vitreous surgeons. Our results have clinical relevance for retinal phototoxicity during the intravitreal use of ICG.

Our results show that the absorption coefficients of short wavelengths in ICG solutions were not significantly greater than those in BSS. They suggest that the intra-

vitreal use of ICG may not result in the higher absorbance of short wavelengths emitted by the endoillumination sources; consequently, an increase in the transmittance of short wavelengths to the sensory retina or retinal pigment epithelium is unlikely. Thus, the use of ICG on the retinal surface probably does not lead to a greater penetration of short wavelengths causing retinal pigment epithelial atrophy. Moreover, it has been recently reported that the hypo-osmolarity of the ICG solution may be responsible for the toxic effect to the retinal pigment epithelium cell, rather than a photochemical effect [12].

Although ICG is physicochemically bound to the species of BSS in the clinical use of ICG during vitrectomy, ICG may also be bound to the plasma components when the breakdown of the blood-retinal barrier occurs, resulting in an alteration of the absorption spectra. Because we speculate that the effect of components other than BSS on the absorption spectra of ICG is minimal, these results may be applied in a practical clinical setting.

In conclusion, the absorption coefficient of wavelengths irradiated by the endoillumination light source in ICG solution is almost the same as that obtained in BSS, and the absorption of short wavelengths is not greater in ICG solution than in BSS. ICG solution placed on the ILM during intravitreal use probably does not enhance retinal photochemical injury during vitrectomy with ICG-assisted ILM removal.

## References

- Burk SE, Da Mata AP, Snyder ME, Rosa RH Jr, Foster RE (2000) Indocyanine green-assisted peeling of the retinal internal limiting membrane. *Ophthalmology* 107:2010–2014
- Dair GT, Ashman RA, Eikelboom RH, Reinholz F, van Saarloos P (2001) Absorption of 193- and 213-nm laser wavelengths in sodium chloride solution and balanced salt solution. *Arch Ophthalmol* 119:533–537
- Engelbrecht NE, Freeman J, Sternberg P Jr, Aaberg TM Sr, Aaberg TM Jr, Martin DF, Sippy BD (2002) Retinal pigment epithelial changes after macular hole surgery with indocyanine green-assisted internal limiting membrane peeling. *Am J Ophthalmol* 133:89–94
- Fox TJ, Wood EH (1960) Indocyanine green: physical and physiological properties. *Proc Mayo Clin* 35:732–744
- Gandorfer A, Messmer EM, Ulibig MW, Kampik A (2001) Indocyanine green selectively stains the internal limiting membrane. *Am J Ophthalmol* 131:388–390
- Ham WT, Mueller HA, Sliney DH (1976) Retinal sensitivity to damage from short wavelength light. *Nature* 260:153–155
- Ham WT, Ruffolo JJ, Mueller HA, Clarke AM, Moon ME (1978) Histologic analysis of photochemical lesions produced in rhesus retina by short wavelength light. *Invest Ophthalmol Vis Sci* 17:1029–1039
- Kadonosono K, Itoh N, Uchio E, Nakamura S, Ohno S (2000) Staining of internal limiting membrane in macular hole surgery. *Arch Ophthalmol* 118:1116–1118
- Kwok AK, Li WW, Pang CP, Lai TY, Yam GH, Chan NR, Lam DS (2001) Indocyanine green staining and removal of internal limiting membrane in macular hole surgery: histology and outcome. *Am J Ophthalmol* 132:178–183
- Michels M, Lewis H, Abrams GW, Han DP, Mieler WF (1992) Macular phototoxicity caused by fiberoptic endoillumination during pars plana vitrectomy. *Am J Ophthalmol* 114:287–296
- Sippy BD, Engelbrecht NE, Hubbard GB, Moriarty SE, Jiang S, Aaberg TM Jr, Aaberg TM Sr, Grossniklaus HE, Sternberg P Jr (2001) Indocyanine green effect on cultured human retinal pigment epithelial cells: implication for macular hole surgery. *Am J Ophthalmol* 132:433–435
- Stalmans P, Van Aken EH, Veckeneer M, Feron EJ, Stalmans I (2002) Toxic effect of indocyanine green on retinal pigment epithelium related to osmotic effects of the solvent. *Am J Ophthalmol* 134:282–285.
- van den Biesen PR, Berenschot T, Verdaasdonk RM, van Weelden H, van Norren D (2000) Endoillumination during vitrectomy and phototoxicity thresholds. *Br J Ophthalmol* 84:1372–1375