光センサを用いた血中色素消失率計測装置とその臨床応用*

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An Instrument Using an Optical Sensor for Measuring Dye Disappearance in Blood and Its Clinical Evaluation for Hepatic Diseases*

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An instrument to measure hepatic function was developed as a medical application of optoelectronic technology. The study of Indocyanine green (ICG) clearance in blood is clinically used for the estimation of hepatic function. Multiple blood samplings, however, subject the patients to mental and physical stress. Furthermore, although it is necessary with this method to correctly measure the blood sampling time after ICG injection, it is difficult to accurately measure this time in an actual test. Thus, a measuring method without blood sampling has been desired. Although some preliminary studies employing a non-invasive method were undertaken, there were still problems for clinical usage. Therefore, in this paper, a description of an instrument for the non-invasive measurement of ICG clearance using an optical sensor attached to the fingertip is presented, and a calibration method before ICG injection is proposed to solve the problem of changing blood volume. Next, the results of the clinical evaluation of 470 cases are presented. Using this newly developed instrument, the values of ICG tests correlated well with the values calculated from the conventional blood sampling method.

1. Introduction

For the estimation of hepatic function, Indocyanine green(ICG)¹⁾ is widely used as a sensitive tracer because the plasma disappearance rate varies from hepatic blood volume, and dye uptake and excreting function of hepatic cells. The principal characteristics of ICG are very low toxicity, confinement to the vascular compartment because of binding to plasma proteins, rapid excretion from liver and presence of an absorption maximum near the isobestic point of hemoglobin⁵⁾. In particular, the study of ICG clearance rate in blood is one of the best methods for evaluating hepatic diseases such as liver cirrhosis^{2),3)}. In 1969 a reference method using ICG clearance rate was established by the Japanese Society of Gastroen-

terology⁴⁾. Clinically, multiple blood samplings have been necessary to determine the values for the ICG tests⁶⁾ such as plasma disappearance rate and 15-minute retention ratio after intravenous ICG injection which subject the patients to mental and physical stress. Furthermore, although it is necessary with this method to correctly measure the blood sampling time after the ICG injection, it is difficult to accurately measure this time in an actual test. Accordingly, a measuring method without blood sampling has been desired. Non-invasive methods were tried to develop by using a spectrophotometric measurement of the ear lobe^{7),8)}. Although the spectrophotometric methods called the ear piece method, were developed as ICG tests without blood sampling, fitting and stabilization of the optical sensor presents some difficulties. To eliminate those problems, a new method by measuring the transmittance light through the tip of the index finger before and after ICG injection was proposed. This paper describes this newly developed instrument and its evalua-

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Fig. 1 Absorption spectra of hemoglobin and indocyanine green(ICG)

tion.

2. Principle of measurement

2.1 Optical characteristics of ICG

After its introduction, ICG soon came into general use for dye dilution tests. The principal advantage of the dye used *in vivo* was the presence of an absorption maximum near the isobestic point of hemoglobin. Absorption spectra of ICG and hemoglobin are shown in Fig. 1. In human tissue, hemoglobin, which is contained in the blood, is the major absorbance factor. The spectrum of hemoglobin changes depending on its oxygen saturation but the absorption maximum of ICG is close to this isobestic point. Therefore, ICG concentration can be measured *in vivo* at 805 nm unaffected by hemoglobin oxygen saturation.

2.2 ICG test using blood sampling

Clinically, the ICG tests are standard methods for the patients with hepatic diseases. The tests are conducted with the patient in a supine position in a fasting state. Blood is sampled before intravenous ICG injection. In general, one blood sampling is performed as a reference. After intravenous injection of ICG, blood sampling is also performed three times after of 5, 10 and 15 minutes. Blood plasma is separated upon coagulation of a blood clot so that the absorbance at 805 nm is measured through a spectrophotometer to obtain ICG concentration values in the blood plasma from a previously obtained line, corresponding to ICG concentration in blood vs. absorbance. These ICG concentration values in the blood plasma are plotted on the ordinate of semilogarithmic scale with time recorded on the abscissa because the disappearance is expected to follow first-order kinetics with respect to the time

after ICG injection. The plasma disappearance rate, $K[\text{min}^{-1}]$ is calculated using the formula;

$$C_t = C_o \cdot \exp(-K \cdot t) \quad (1)$$

$$K = \frac{\log C_o - \log C_t}{t} \quad (2)$$

where t is time in minutes after ICG injection, C_t is ICG concentration in the blood plasma after t minutes and C_o is the calculated initial ICG concentration in the plasma. When the ICG dosage is 0.5 mg per kilogram of body weight, the 15 minute retention ratio R_{15} as a clinical estimation value of hepatic function is defined as follows;

$$R_{15} = \frac{C_{15}}{C_o} \cdot 100 [\%], \quad (3)$$

usually, C_o in eq. (3) is clinically determined to be 1.00.

2.3 Prototype of continuous method

A dichromatic ear densitometer measured the difference of the transmitted light at 800 and 900 nm to derive the ICG concentration (S_g) as follows;

$$S_g = K_g \cdot (\log T_{800} - \log T_{900}), \quad (4)$$

where K_g is a constant that depends on the ICG absorption coefficient. T_{800} and T_{900} are the transmitted lights at 800 and 900 nm respectively. The value $\log T_{800}$ represents absorbances of ICG and tissue including hemoglobin, but the value $\log T_{900}$ represents an absorbance of tissue because of ICG's absorption spectrum. However, the output measured by eq. (4) fluctuates due to influence from the changing blood volume at the measuring site. Considering this problem, the following relationship was derived⁸⁾,

$$S_g = \left\{ \frac{\log \left(\frac{I_{800}}{T_{800}} \right)}{\log \left(\frac{I_{900}}{T_{900}} \right)} - K_b \right\} \cdot K_g \quad (5)$$

where K_b is the constant value of the bloodless state. In this method, before ICG injection, the ear lobe is pressed by the ear piece of the dichromatic ear densitometer in order to obtain measurements of I_{800} and I_{900} , the bloodless transmitted lights at 800 and 900 nm. Next, measurements of the normal blood-containing state is obtained by releasing the pressure on the earlobe and measuring T_{800} and T_{900} , the transmitted lights at the same two wavelengths. In actuality, the values I_{800} , I_{900} and K_b are treated as constant

values. Using the two conditions described above as references, T_{800} and T_{900} , transmitted lights of the former normal state after ICG injection, are measured to calculate S_g value. As a result, it is reported that the influence of the change of blood volume can be compensated for using this method. However, to obtain correct ICG disappearance curve, common problems that exist in conventional ear densitometry must be addressed:

- (a) because using a xenon or halogen lamp as a light source heats the ear piece, more than 20 minutes idling time is necessary to stabilize the densitometer, but since the ear lobe is warmed, the blood volume gradually increases and hemoglobin is oxygenated causing the change of absorbance at 900 nm as a reference.
- (b) the ear piece frequently slips down because of its weight, but the blood volume decreases when it is attached tightly to prevent slipping, and
- (c) to get enough absorbance of ICG from the ear lobe, heating or some other method is necessary to increase blood flow.

3. The newly developed measuring algorithm

Relationship between the transmitted lights at 810 and 940 nm before injection of ICG are expressed as:

$$\log T_{810} = A_{810} + A_{810}^{\text{ICG}} \quad (6)$$

$$\log T_{940} = A_{940} \quad (7)$$

where T_{810} and T_{940} : transmitted lights through the fingertip at 810 and 940 nm, A_{810} and A_{940} : are the absorbances of tissue and blood at 810 and 940 nm.

Extracting absorbance information on ICG is done by seeking A_{810}^{ICG} from eq. (6). However, changes in the ICG concentration contain a large noise component. This is because the absorbance of the blood varies greatly with changes in the blood volume in the end of the finger, thus causing the noise component.

In order to remove this noise component, a wavelength at which ICG does not have absorbance is used as shown in eq. (7). The relationship between the absorptions of the two wavelengths by the blood and tissue is shown by the following equation experimentally before the intravenous injection of ICG, which establishes calibration constants between the wavelengths of the

transmitted light.

$$\log T_{810} = \alpha \cdot \log T_{940} + \beta \quad (8)$$

Here, α and β are constants decided by the least square method at the time calibration is actually performed. This equation means that there is a linear relationship between $\log T_{810}$ and $\log T_{940}$ because the absorbances 810 and 940 nm depend only upon the changing of blood volume before ICG injection. In practice, eq. (8) is obtained by greatly changing the amount of blood volume in the end of the finger by raising and lowering the hand to which the ICG sensor has been attached. After intravenous injection of ICG, the calibration data from eq. (8) is used to calculate the blood and tissue absorbance of eq. (6) from eq. (7), and the ICG absorbance A_{810}^{ICG} is calculated by subtracting this. Therefore, the ICG concentration, S_g , is defined as:

$$S_g = \{\log T_{810} - (\alpha \cdot \log T_{940} + \beta)\} \cdot K_g \quad (9)$$

The value S_g is constantly zero before the injection of ICG, if the blood volume changes, and is defined as the base line of the disappearance curve. The time change of S_g is expressed as:

$$S_g = S_o \cdot \exp(-K \cdot t) \quad (10)$$

where t represents the elapsed time in minutes after injection of ICG, and S_o represents the S_g value at $t=0$. Further, R_{15} is expressed as:

$$R_{15} = \exp(-K \cdot 15). \quad (11)$$

4. Description of the system

4.1 Optical sensor

The ICG clearance meter has light emitting diodes (LEDs) that emit at 810 and 940 nm, and a near-infrared silicon photodiode used as a photodetector. The 810 nm wavelength is very close to 805 nm isobestic wavelength and this transmitted light variation due to hemoglobin oxygen saturation is negligible. In addition, the optical absorption by ICG is quite high at 810 nm, but quite low at 940 nm. Two LEDs are mounted on one small, glass-epoxy base. The LED base and photodiode are mounted on a flexible printed circuit (FPC). This FPC is molded by casting polyurethane for electric insulation and mechanical durability. Clinically, as shown in Fig. 2, the optical sensor is fixed on the tip of the index finger by using a soft adhesive PVC tape to prevent blood congestion and permit this sensor to be at-

Fig. 2 Optical sensor attached to the fingertip

tached to a finger tip with any shape.

4.2 The hardware system

Fig. 3 shows a hardware block diagram. The LEDs are pulsed in order to obtain higher intensity output, to eliminate DC drift problems and to avoid LED overheating. The light transmitted through the finger is detected by the photodiode. The resulting signal from the photodiode is amplified and enters the sample/hold circuits where the difference of the transmitted lights at each wavelength, and the background are synchronously sampled and held. The sampled DC signal is digitized for computer processing, then latched in order to be taken into the micro-computer synchronously.

Fig. 3 Block diagram of the hardware system

4.3 The system characteristics

This instrument measures the change in the blood ICG concentration over time, and it is necessary that the output of the optical sensor reflects the ICG concentration. Therefore, in order

to evaluate the basic performance of this instrument, a standard solution equivalent to the blood ICG concentration during ICG test is prepared. The optical sensor was attached to an optical cuvette filled with the standard solution and sought the relationship between the ICG concentration and the output voltage, S_g [V], of the sensor. The results, as shown in Fig. 4, confirmed that the output of the sensor demonstrated a linear relationship with the ICG concentration.

4.4 Results of the proposed calibration

It is necessary to compensate for the change of blood volume which has an important effect upon baseline stability of the ICG clearance. Fig. 5

Fig. 4 The linearity between ICG concentration and output voltage

Fig. 5 Baseline Stability

shows an evaluation of the baseline stability, where Fig. 5(a) shows time changes of transmitted lights (T_{810} and T_{940}) in $-\log(T)$ for 15 minutes in a resting state without the injection of ICG. Using a conventional method based on the eq. (5), the baseline fluctuates largely as characteristic 'old method' in Fig. 5(b). On the other hand, when the change of blood volume is corrected with the proposed method in this paper according to eq. (9), the baseline is substantially and practically more stable as shown by characteristic 'new method' in Fig. 5(b).

5. Clinical evaluation

5.1 Method

Hepatic clearance studies were obtained in 16 healthy adult subjects who had histologic evidence of a normal hepatic and 454 patients with biochemical evidence of hepatic diseases (103 chronic hepatitis, 134 liver cirrhosis, 67 hepatoma, 11 acute hepatitis and 139 with other diseases). The optical sensor was attached to the tip of the index finger using adhesive tape. Each patient was injected rapidly without force in the antecubital vein with 0.5 mg of ICG per kilogram of body weight. Results were analyzed statistically by the Student's t test.

5.2 Result

(1) Comparison with blood sampling method

The correlation between values from the blood sampling method and those of the finger-piece method was examined. A correlation equation for K indicated $y = 0.816x - 0.002$ ($r = 0.889$) (Fig. 6). The correlation equation for R_{15} showed $y = 1.1x + 6.4$ ($r = 0.951$) (Fig. 7).

(2) Values in hepatic diseases

K and R_{15} for various hepatic diseases measured by the finger piece method were examined. All values were expressed by the mean $\pm$ the standard deviation. Values of K were 0.147 ± 0.022 in the healthy control group, 0.156 ± 0.051 in the acute hepatitis group, 0.118 ± 0.035 in the chronic hepatitis group and 0.071 ± 0.036 in the hepatic cirrhosis group. A significant difference ($p < 0.001$) was obtained between the chronic hepatitis group and the control group. Also, there was a significant difference ($p < 0.001$) between the liver cirrhosis group and the control group.

Values of R_{15} were $11.63 \pm 3.69\%$ in the control group, $13.36 \pm 11.94\%$ in the acute hepatitis group, $19.29 \pm 8.98\%$ in the chronic hepatitis group and $39.39 \pm 19.16\%$ in the liver cirrho-

Fig. 6 K values between conventional blood sampling method and noninvasive method

Fig. 7 R_{15} values between conventional blood sampling method and noninvasive method

sis group. The chronic hepatitis group showed a significant difference ($p < 0.001$) from the control group and from the liver cirrhosis group. In addition, the liver cirrhosis group showed a significant difference ($p < 0.001$) from the control group. A significant difference was not observed in the liver cirrhosis group between cases with or without primary hepatic carcinoma.

6. Discussion

In this paper, a description of an instrument for the continuous non-invasive measurement of both K and R_{15} and the results of the clinical evaluation for about 470 patients were presented. The authors propose a biocalibration method before ICG injection particularly *in vivo* measurement, to solve the problem of changing blood volume, satisfactory results were consequently obtained. It

is interesting to compare our method with the previous study. For example, Yokosuka et al., settled an idling time to stabilize the change of blood volume. For deleting artifacts, however, this method can be considered as a passive way in comparison with the positive method as explained in this paper. It is not necessary to settle an idling period in the proposed method. Additionally, in the clinical evaluation, the non-blood-sampling method was found to be more convenient because there was no waiting time and no infection, which often results from blood sampling. Another main advantage of this method is that a continuous ICG disappearance curve is obtained. The disappearance curve makes it possible for the progress of hepatic lesions to be diagnosed clearly without calculating K and R_{15} . Moreover, the curve would be helpful for researchers who are interested in the kinetics of ICG, when, they construct or analyze compartment models^{9),10)} based on the compartment analysis.

7. Conclusion

An instrument for an ICG test of hepatic function was developed. A high correlation between the values obtained by this method and by the blood sampling method was observed, showing that the ICG test by the finger-piece method is considered to be a useful and sensitive method for clinical use.

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