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Application of a Non-invasive System for Monitoring Plasma Indocyanine Green Dynamics in Rats

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Summary. **A non-invasive indocyanine green (IGG) monitoring system was applied to rats to estimate the plasma disappearance rate of ICG (ICG-K). The optical sensor for monitoring the ICG concentration was attached to the skin of the forepaw of the posterior leg. When ICG 0.5 mg/kg (200 μl) was injected into the jugular vein as a bolus, a plasma disappearance curve for ICG was obtained. The curve shifted downwards according to the decrease in the ICG dosage, and reproducible values in ICG-K were obtained when 0.25 mg/kg and 0.5 mg/kg ICG were injected. The curve obtained from the monitoring system was similar to that from the blood sampling method. It was also noted that the coefficients of variation in systemic arterial blood pressure and portal venous blood flow were both within 5 during the 15-min ICG examination.**

These results suggest that this ICG monitoring system is suitable for investigating the ICG metabolism with stable systemic and portal circulation in the rat.

Key words—hepatic dye test, dye metabolism, liver.

INTRODUCTION

The indocyanine green (ICG) test, a reliable method for determining effective hepatic blood flow and hepatocyte function,[1,2] and is also quite useful for estimating the liver function, measuring the blood flow in an extrahepatic shunt and determining the severity of liver disease.[3-7] A non-invasive ICG monitoring system was recently developed, and its usefulness was reported in humans.[8] In this system, an optical sensor for estimating the plasma ICG concentration is attached to the skin of a finger in the hand.

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The present study was designed to investigate whether the ICG monitoring system is applicable to the rat in researching ICG dynamics in the blood.

MATERIALS AND METHODS

Thirty-two male Wistar rats weighing about 250 g were used. The animals were anesthetized with an intraperitoneal injection of pentobarbital sodium (45 mg/kg), and the depth of anesthesia was maintained by intramuscular injection of the same agent (7.5 mg/kg) every 30 min.[9] A small catheter for ICG injection was inserted into the right jugular vein.

An ICG monitoring system (RK-1000, Sumitomo Electric Industries Ltd., Osaka, Japan) was utilized. Plasma ICG concentrations serially expressed between 5 and 15 min were plotted, and the ICG-disappearance rate (ICG-K) was calculated by the least square method. The optical sensor for estimating the plasma ICG concentration was attached to the skin of the forepaw of the posterior leg. Briefly, the dorsal surface of the forepaw was shaved with scissors, then both the dorsal and plantal surfaces of the paw were wiped with a piece of gauze moistened with 70% alcohol. The sensor was fixed to the skin with adhesive tape, but the optical transmitter and receiver areas were not taped. Calibration was performed by moving the leg up and down in a supine position; this maneuver was repeated five times with the hip joint angle above 70°.

Blood for ICG estimation (100 μl) was also withdrawn from the jugular vein, with blood samplings made 3, 5, 7, 10 and 15 min after the ICG had been given. Plasma concentrations of ICG were calculated from the absorbance at 802 nm with a spectrophotometer (Hitachi 105-50, Hitachi, Tokyo).[5]

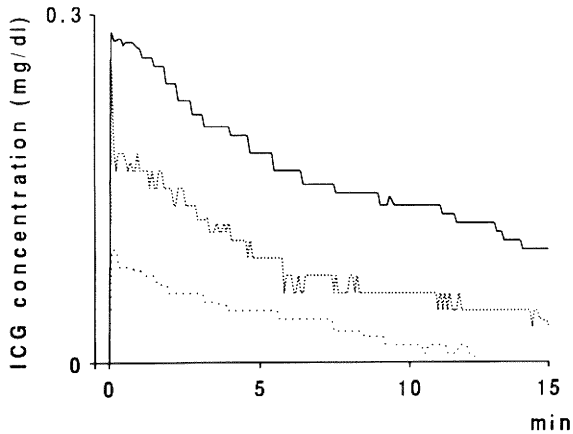

Fig. 1. Representative alterations in plasma disappearance curves of ICG. ICG 0.1 mg/kg (·····) or 0.25 mg/kg (·····) or 0.5 mg/kg (——) was administered. Zero indicates the time of injection.

Portal venous blood flow (PVF) was measured with a transit-time ultrasonic volume flowmeter (Transonic T101, Advance, NY, USA) connected to a 2 mm probe.[9,10] The systemic arterial blood pressure (SAP) was recorded from the right carotid artery.[10]

ICG (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan) was dissolved in the aqueous solvent provided, and was injected into the right jugular vein. ICG 0.5 mg/kg injection was used as a reference for the dose normally used in human subjects,[1,2] and the dose of ICG was changed as required. The amount of one test injection was 200 μl.

The data were ANOVA analyzed, and specific values were evaluated by Duncan's multiple range test. $p<0.05$ was regarded as significant.

RESULTS

The plasma disappearance curves for ICG at three different doses are shown in Fig. 1. The curve shifted downwards as the dose of ICG was decreased, and ICG-K values after 0.25 and 0.5 mg/kg ICG administration were obtained. ICG 0.1 mg/kg failed to estimate ICG-K (Fig. 1 and Table 1A).

When 0.25 and 0.5 mg/kg ICG were injected repeatedly into the same animal, there was good reproducibility in ICG-K (Table 1B).

When ICG plasma disappearance curves obtained by the non-invasive monitoring and blood sampling methods were compared, no significant difference in ICG values was obtained during the 15-min observation period in 0.25 and 0.5 mg/kg ICG administration (Fig. 2).

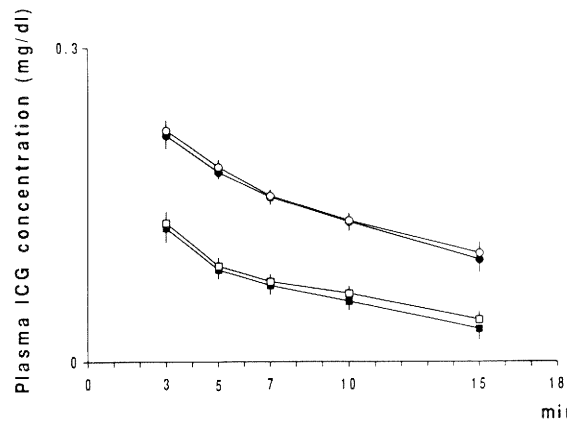

Fig. 2. Plasma disappearance curves of ICG. Two different doses of ICG (□——■, 0.25 mg/kg; ○——●, 0.5 mg/kg) were given, and ICG concentrations were estimated by the non-invasive monitoring (○——□) and blood sampling (●——■) methods. Zero shows the time of injection. Values are the means±SEM (n=6).

Table 1A. ICG-K after three doses of ICG administered to different rats

ICG (mg/kg)	ICG-K
0.5	0.137±0.181
0.25	0.171±0.020
0.1	Not calculated

Values are the means±SEM (n=6)

Table 1B. ICG-K after two doses of ICG injected into the same rat

ICG (mg/kg)	ICG-K
0.5	0.141±0.090
0.25	0.168±0.052

Values are the means±SEM (n=5)

The volume (200 μl) of the ICG injection caused no meaningful change in systemic or portal circulation; the coefficients of variation (mean±SEM, n=5) in SAP and PVF during the 15-min ICG examination were 0.6±2.7 and 4.4±1.9, respectively (Fig. 3).

DISCUSSION

We found that the ICG monitoring system proposed here is applicable to the rat in researching ICG dynamics.

Although application of the ICG test to rats has

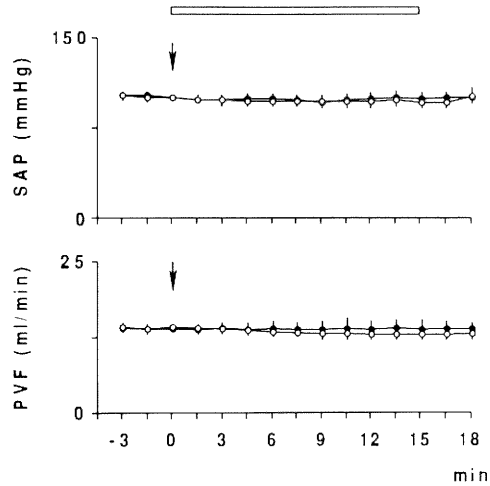

Fig. 3. Changes in SAP and PVF after ICG injection. ICG (0.25 mg/kg, 200 μg) (●) was injected. No ICG injection was done as the control (○). The arrow shows the time of injection, and the rectangle the duration of examination. Values are the means±SEM (n=5).

previously been reported, it is not easy to draw blood samples repeatedly.[11-13] In this non-invasive method, no blood sampling is required and the maneuver is simple.

Repetitive examination of ICG-K in the same rat is also difficult when ICG tests are done with blood sampling methods.[11-13] With this system, ICG-K could be estimated repeatedly in the same animal quite easily. When 0.25 and 0.5 mg/kg ICG tests were conducted, there was good reproducibility in ICG-K values. Since ICG 0.25 mg/kg injection produced good reproducibility in ICG-K and a smaller dose of ICG caused less hepatotoxicity,[14,15] an ICG dose of 0.25 mg/kg may be suited to rats.

Because the disappearance slope for ICG administration is similar in both non-invasive monitoring and blood sampling methods, the effectiveness of ICG clearance tests by both methods is nearly the same.

The volume effect of the ICG injection on systemic circulation could be neglected because fluctuation in SAP during the 15-min ICG examination was very small and similar ICG-K values were recorded. In addition, stable hepatic blood flow could be expected during the ICG examination, though blood sampling methods substantially influence hepatic and systemic circulation.[11-13]

Although prostaglandin E_1 and ursodesoxycholic acid increase hepatic blood flow by dilating hepatic vessels,[6,9,16] a smaller dosage of these agents raised ICG-K without any changes in PVF (unpublished data, Fujita and Sakaguchi). The combination of ICG and the blood flow monitoring system proved valid as a means of estimating ICG incorporation into the hepatocytes under fixed hepatic circulation. Moreover, analysis could be done to a fine degree because recording was serial. This system may be widely applicable in this field.

The variation in SAP and PVF observed could be excluded in determining rat ICG-K because almost the same ICG-K values were obtained. However, changes in SAP are reflected in the hepatic arterial blood flow,[1,2] and a close relationship between the hepatic arterial circulation and ICG-K has been pointed out.[1] Further study on this point is necessary.

These observations lead us to conclude that the ICG monitoring system is suitable and proper for use in investigating the rat ICG metabolism.

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