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Utilization of Low Dose Indocyanine Green Test for Evaluating Liver Function

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Summary. **Liver function was examined in 60 patients with a 0.1 and 0.5 mg/kg intravenous bolus of indocyanine green (ICG). When plasma ICG concentrations were measured 3, 5, 7, 10 and 15 min after time zero, the removal slope for ICG was consistently exponential for the 15-min observation period. The slope was similar between the two ICG administrations in log scale. When disappearance rates of ICG 10 min after time zero between the 0.1 and 0.5 mg/kg tests were compared, a significant correlation ($p<0.01$) was obtained. Serum bilirubin fluctuation ranging from 0.4 to 3.7 mg/dl had no meaningful effect on the efficacy of the 0.1 mg/kg ICG test.**

These results suggest that an ICG 0.1 mg/kg test is accurate enough to estimate the liver function, and has potential value for clinical application.

INTRODUCTION

The indocyanine green (ICG) test is quite useful for determining hepatic blood flow and liver functions.[1-4] In resecting hepatocellular carcinoma, the remaining liver function must be assessed after hepatectomy to avoid posthepatectomy liver failure due to excessive resection.[5,6] The dose of ICG sufficient to estimate liver function is conventionally judged to be 0.5 mg/kg as an intravenous bolus when decision in the test is made after 15-min observations. However, it has also been shown that ICG possesses hepatotoxicity where a dose-dependent and cumulative effect is observed.[7,8] These reports suggest that an ICG test with a smaller dose is preferable in order to prevent the toxic effect, especially when massive liver resection and repeated assessment are required.

The present study was designed to investigate whether the result of an intravenous 0.1 mg/kg ICG bolus test is compatible with that of the conventional 0.5 mg/kg ICG test for estimating liver function.

SUBJECTS AND METHODS

Subjects

A total of 60 patients were tested. Clinical diagnoses of subjects are shown in Table 1, with 11 of the 60 subjects receiving hepatectomy.

Study design

ICG tests were performed in the morning before breakfast. Both 0.1 and 0.5 mg/kg ICG tests were done at approximately 30-min intervals in the same subjects. They were arranged in order of increasing dosage. The tests were conducted repeatedly before

Table 1. Subjects who received ICG tests

Diagnosis	No. of subjects
Hepatoma with cirrhosis	11
Liver cirrhosis	6
Hepatoma without cirrhosis	7
Cholangiocellular carcinoma	6
Metastatic tumor of the liver	6
Gallbladder carcinoma	9
Bile duct carcinoma	5
Pancreatic carcinoma	7
Hepatolithiasis	1
Hemangioma of the liver	1
Multiple liver cyst	1
Total	60

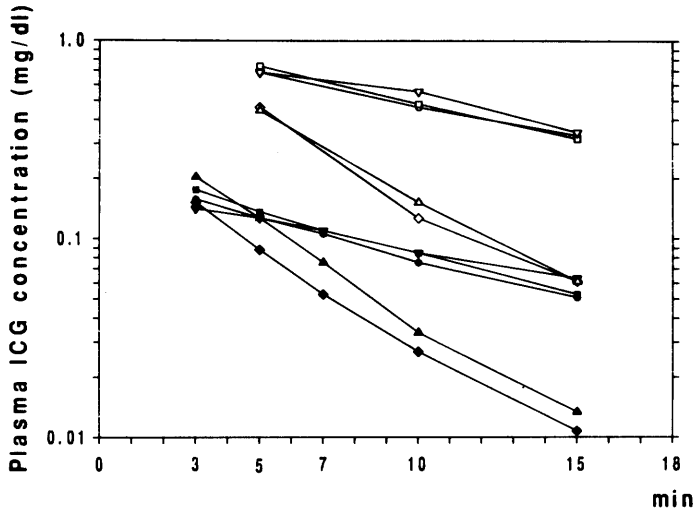

Fig. 1. Plasma removal slope for ICG in five subjects. Two different doses of ICG (open mark, 0.5 mg/kg; filled mark, 0.1 mg/kg) were given to the same subjects.

and after operative treatments in the same subjects.

Administration and estimation of ICG

ICG was dissolved in the aqueous solvent provided, and 0.1 mg/kg and 0.5 mg/kg body weight were given as an intravenous bolus. ICG was injected into the cubital vein in the forearm, and blood was withdrawn from the cubital vein on the opposite side. Time zero was established when the bolus had been given, and blood samplings were made 3, 5, 7, 10 and 15 min after time zero. Plasma concentrations of ICG were calculated from the absorbance at 802 nm with a spectrophotometer (Hitachi 105-50, Hitachi, Tokyo).[3]

Estimation of liver functional parameters in the blood

The blood samples for chemical analysis were obtained from the cubital vein, were cooled immediately with ice water and centrifuged at 2,200 rpm for 20 min. The separated serum was then stored at −20℃ until measurement of the following parameters with an autoanalyzer (Hitachi 736, Hitachi, Tokyo):[5] total protein (TP, Biuret method); albumin (Alb, Bromcrezol green method); glutamic oxaloacetic transaminase (GOT, Ultraviolet method); glutamic pyruvic transaminase (GPT, Ultraviolet method); alkaline phosphatase (Alp, Bessey-Lowry method); γ-glutamyltranspeptidase (γGTP, Ultraviolet method); total bilirubin (TB, Azobilirubin method); total cholesterol (TC, cholesterol oxidase colorimetric method).

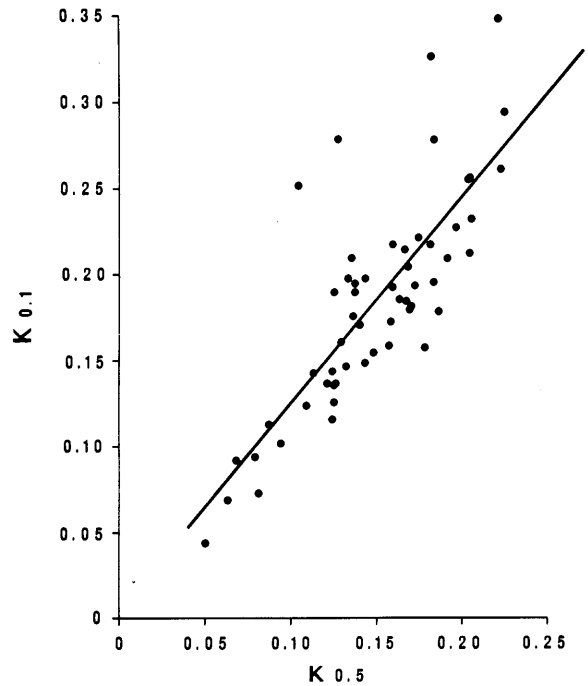

Fig. 2. Correlation in the disapperance rate for ICG between 0.1 (Y, $K_{0.1}$) and 0.5 (X, $K_{0.5}$) mg/kg tests. The regression line is $Y = 1.2098X + 0.004$, and the correlation is significant, $p < 0.01$.

Statistical analysis

The data were evaluated by *t*-test. Line regression analysis was also used.

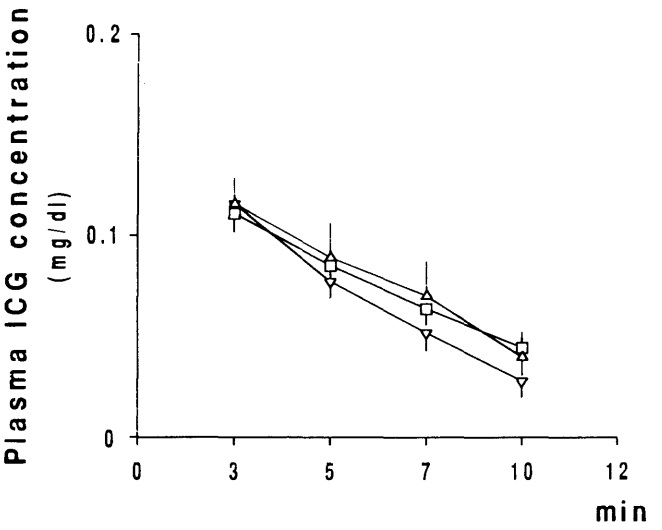

Fig. 3. Effects of bilirubin on the plasma removal slope for ICG 10 min after time zero. ICG 0.1 mg/kg tests were done at three different concentrations of bilirubin (△, n=8, about 0.4 mg/dl; □, n=8, about 1.0 mg/dl; ▽. n=8, about 3.7 mg/dl). Values are the means ± SEM.

Table 2. Serum liver functional parameters before (I) and 2 weeks after (II) operative treatments

	I	II
TP (g/dl)	7.7 ± 0.2	7.0 ± 0.2[a]
Alb (g/dl)	3.6 ± 0.1	3.3 ± 0.4
GOT (IU/l)	61 ± 16	62 ± 9
GPT (IU/l)	95 ± 31	100 ± 21
Alp (IU/l)	438 ± 116	397 ± 71
γGTP (IU/l)	206 ± 67	93 ± 19[a]
TB (mg/dl)	1.09 ± 0.20	0.93 ± 0.06
TC (mg/dl)	182 ± 11	123 ± 7[a]

Values are the means ± SEM (n=11). [a]$p<0.01$ vs I.

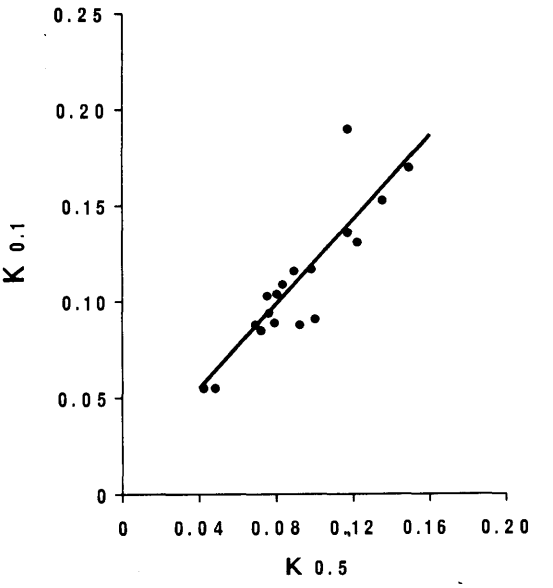

Fig. 4. Correlation in the disapperance rate of ICG between 0.1 (Y, $K_{0\cdot1}$) and 0.5 (X, $K_{0\cdot5}$) mg/kg tests in the subjects with cirrhosis. The regression line is $Y=1.2233X+0.007$, and the correlation is significant, $p<0.01$.

RESULTS

The removal curve for ICG in the blood was consistently exponential for the 15-min observation when 0.1 and 0.5 mg/kg ICG were administered (Fig. 1). The curve was similar between the two ICG administrations in log scale.

When the disappearance rates for ICG 10 min after administration between the 0.1 and 0.5 mg/kg ICG tests were compared, a significant correlation between the two components was obtained (Fig. 2). The regression coefficient (r) was +0.796.

Fig. 3 shows the results of the ICG test of 0.1 mg/kg dose performed in three groups with different bilirubin concentrations. Each group comprises eight patients with serum bilirubin concentrations of 0.4± 0.0, 1.0±0.0 and 3.7±0.6 mg/dl, respectively. When

ICG disappearance rates were compared among the three groups at 3, 5, 7 and 10 min after time zero, no significant difference was observed. Thus, a serum bilirubin concentration of less than 4 mg/dl might not greatly affect the efficacy of this test.

The serum concentrations of substances indicating hepatic function were not significantly different before and after the operation (Table 2).

A significant correlation in the disappearance rate of ICG was obtained between the 0.1 and 0.5 mg/kg ICG tests when they were applied to the subjects with cirrhosis (Fig. 4). The regression coefficient (r) was + 0.887. It was also noted that repeated tests with ICG provided good reproducibility in the rate of disappearance; the coefficients of variation (mean±SEM, n=8) for the 0.1 and 0.5 mg/kg ICG were 8.9±1.8 and 6.1±1.4 in the same subjects, and no significant difference was obtained.

No important complications were observed in the tests.

DISCUSSION

We found that the removal slope for 0.1 mg/kg ICG application was similar to that for 0.5 mg/kg during the 15-min observation (Fig. 1). This could mean that the effectiveness of the 0.1 mg/kg ICG test was equivalent to that of the 0.5 mg/kg ICG test in estimating liver function when the slope was constructed from values obtained during the 10 min observation.

The finding that almost the same slope line was reproduced in the 0.1 and 0.5 mg/kg ICG applications on the same subjects (Fig. 1) suggests that the 0.1 mg/kg ICG test is valid in evaluating liver function. Support for this idea is also provided by the fact that there was a good correlation between the 0.1 and 0.5 mg/kg ICG tests in the disappearance rate (Fig. 2).

It has been shown that the bilirubin in the blood competitively interacts with the absorption of ICG in the liver parenchymal cells: the higher the bilirubin concentration, the lower the ICG disappearance rate.[2,3] In this study, no significant difference in the removal slope for ICG was obtained even though there were three different concentrations of bilirubin within about 4 mg/dl. This could be interpreted as meaning that the 0.1 mg/kg ICG test is effective even when bilirubin fluctuates with the diseases listed in Table 1.

ICG has been shown to have hepatotoxic effects: it changes the permeability of the hepatocyte, adversely affecting the liver cytochrome system, and inhibits bile salt-independent bile formation.[7,8] In this study, no deteriorating change in serum parameters of liver dysfunction was seen following operative treatment in spite of the repeated ICG application (Table 2). However, this does not mask the merit of using a smaller dose of ICG.

A good correlation in the ICG disappearance rate between the 0.1 and 0.5 mg/kg ICG tests was obtained in the subjects with cirrhosis (Fig. 4); good reproducibility was also obtained in the same subjects. It appears that the 0.1 mg/kg ICG test is suitable for clinical use, especially in the case of hepatic sclerotic impairment.

The ICG test has been shown to be one of the most reliable examinations in predicting posthepatectomy liver failure.[9-11] The test has also recently been applied to the assessment of liver transplantation.[12] In these situations, the smaller dose ICG test proposed is appropriate for the damaged liver in which the functional reserve is extremely reduced.

These observations led us to conclude that the 0.1 mg/kg ICG test is sufficiently accurate as a liver function test, and is suitable for clinical application.

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REFERENCES

1) Leevy CM, Mendenhall CL, Lesko W, Howard MM: Estimation of hepatic blood flow with indocyanine green. *J Clin Invest* **41**: 1169-1179, 1962.
2) Cherrick GR. Stein SW. Leevy CM, Davidson CS: Indocyanine green: Observations on its physical properties, plasma decay, and hepatic extraction. *J Clin Invest* **39**: 592-600, 1960.
3) Paumgartner G, Probst P, Kraines R, Leevy CM: Kinetics of indocyanine green removal from the blood. *Ann NY Acad Sci* **170**: 134-147, 1970.
4) Kawasaki S, Sugiyama Y, Iga T, Hanano M, Sanjo K, Beppu T, Idezuki Y: Pharmacokinetic study on the hepatic uptake of indocyanine green in cirrhotic patients. *Am J Gastroenterol* **80**: 801-806, 1985.
5) Nagasue N, Inokuchi K, Iwaki A, Yukaya H, Kanashima R, Saku M, Kobayashi M: Remnant liver function during surgery for extensive hepatic resection. *Jpn J Surg* **9**: 125-131, 1979.
6) Mizumoto R, Kawarada Y, Noguchi T: Preoperative estimation of operative risk in liver surgery, with special reference to functional reserve of the remnant liver following major hepatic resection. *Jpn J Surg* **9**: 343-349, 1979.
7) Horak W, Grabner G, Paumgartner G: Inhibition of bile salt-independent bile formation by indocyanine green. *Gastroenterology* **64**: 1005-1012, 1973.

8) Laperche Y, Oudea MC, Lostanlen D: Toxic effects of indocyanine green on rat liver mitochondria. *Toxicol Appl Pharmacol* **41**: 377-387, 1977.
9) Okamoto E, Kyo A, Yamanaka N, Tanaka N, Kuwata K: Prediction of the safe limits of hepatectomy by combined volumetric and functional measurements in patients with impaired hepatic function. *Surgery* **95**: 586-592, 1984.
10) Takasaki T, Kobayashi S, Suzuki S, Muto H, Marada M, Yamana Y, Nagaoka T: Predetermining postoperative hepatic function for hepatectomies. *Int Surg* **65**: 309-313, 1980.
11) Yamanaka N, Okamoto E, Kuwata K, Tanaka N: A multiple regression equation for prediction of post-hepatectomy liver failure. *Ann Surg* **200**: 658-663, 1984.
12) Clements D, McMaster P, Elias E: Indocyanine green clearance in acute rejection after liver transplantation. *Transplantation* **46**: 383-385, 1988.