RELATION OF THE SIZE OF FUNCTIONAL HEPATIC CELL MASS TO THE CLEARANCE OF INDOCYANINE GREEN

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ABSTRACT

The correlation between fractional clearance (K) of indocyanine green (ICG) and size of hepatic cell mass altered by functional hepatectomy as well as the degree of hepatic damage induced by carbon tetrachloride (CCl_4) was investigated in the dog. Less than 20% functional hepatectomy produced only a minimal decrease in K of ICG probably due to the potential functional reserve of the liver, while in dogs with more than 70% functional hepatectomy, K of ICG was remarkably decreased. K of ICG was correlated highly with the magnitude of functional hepatectomy, i.e. the size of remaining functional hepatic cell mass. Large doses of CCl_4 induced remarkable decreases in K of ICG, being correlated positively with the degree of hepatic cell necrosis, and CCl_4 in smaller doses rather activated the hepatic cell function as reflected by higher K values. It was concluded that K of ICG was a reliable indicator of function of hepatic cell mass in acute or subacute damage of the hepatic cell.

INTRODUCTION

Indocyanine green (ICG) is a tricarbocyanine dye introduced by Fox *et al.*[1] for the purpose of measuring cardiac output. This compound is rapidly bound to β-lipoprotein[2] and albumin[3~4], as rapidly distributed and stabilized in plasma following its intravenous injection. This dye is more useful than sulfobromphthalein (BSP) in the evaluation of hepatic function on account of its complete biliary excretion and the absence of enterohepatic circulation.[5] Since 1963, the fractional clearance (K) of ICG has been used as one of the indices in the differential diagnosis between cirrhotic and non-cirrhotic portal hypertension (Fig. 1) and as one of the criteria for determining operability of portal hypertension of cirrhotic variety (Fig. 2). Since K of ICG is considered to be indicative of the amount of functional hepatic cell mass as well as the degree of hepatocellular damage, in the present study, the correlation between K of ICG and the size of the hepatic cell mass by functional hepatectomy as well as the degree of hepatic cell damage by CCl_4 was experimentally investigated in the dog.

MATERIAL AND METHODS

Adult mongrel dogs of both sexes weighing 7 to 13 kilograms were used in this study. ICG (Diagnogreen Injection, Daiichi Seiyaku Ltd.) solution was prepared just prior to the experiment by dissolving 25mg of ICG (2.5mg/ml) in 10ml of distilled water. Each dog was fasted for a minimum of 12 hours, and anesthetized with thiopental sodium (Ravonal®)

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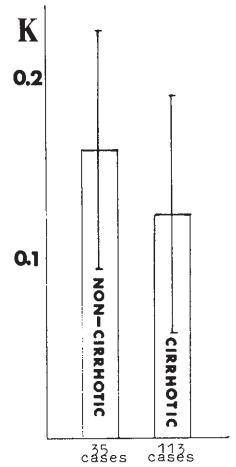

Fig. 1

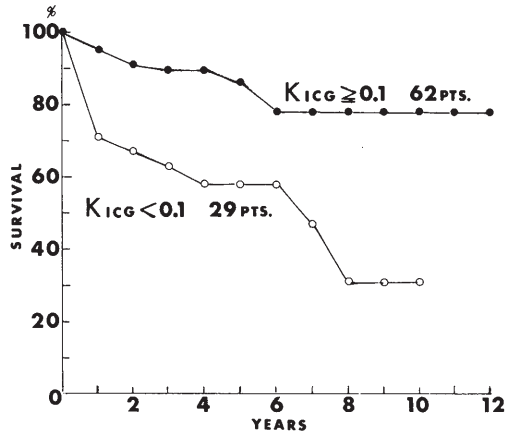

Fig. 2

Fig. 1. Fractional clearance (K) of ICG (mean ± S.D.) in non-cirrhotic and cirrhotic portal hypertension ($p < 0.01$).

Fig. 2. Postoperative survival curve in cirrhotic portal hypertension.

200 – 500mg (i.v.). The femoral vein was cannulated with a polyethylene tube and was kept open by dripping saline solution. After obtaining control blood sample, ICG was injected into the tongue vein, in a dose of 0.25mg* per kilogram of body weight. Subsequent blood sampling was made from the cannula in the femoral vein 3, 6, 9 and 12 minutes after injection of ICG. One ml of plasma was diluted with two ml of normal saline and ICG concentrations were determined spectrophotometrically (Hitachi 181 type) at wave length of 805mμ.

After a single intravenous injection of the indicator, the decay of plasma concentration follows an exponential law: $C(t) = C_0 . e^{-kt}$, where C(t) is the concentration at the time of t; C_0, the theoretical concentration at the time of zero; e, the base of Naperian logarithm; k, fractional clearance or a constant. K will be calculated by the following formula: $K = \frac{1n\ 2}{t^{1/2}} = \frac{0.693}{t^{1/2}}$, $t^{1/2}$, the time when the plasma concentration has decayed to half of C_0.

STUDIES IN FUNCTIONALLY HEPATECTOMIZED DOGS: Functional hepatectomy implies a procedure in which given liver lobes are functionally handicapped by simply ligating the pertinent portal and arterial blood vessels as well as bile ducts without removing the liver lobes (Fig. 3). Functional hepatectomies of varying scales were performed in 29 dogs. First, control K of ICG was estimated in dogs which were simply anesthetized and not yet surgically intervened, and laparotomy was made one hour later. The hepatic portal region was infiltrated with 1% lidocaine (Xylocaine®) in order to block any neural reflex which might possibly be caused by ligation of blood vessels or bile ducts. Functional

* A preliminary experiment was done to determine the optimal ICG dose by testing with various doses of ICG, and it was found that K value of ICG given in a dose of 0.25mg/kg reflected the hepatic function in dogs most sensitively. It was also confirmed that hepatic removal of ICG was exponential during the 12 min. period with nearly perfect linearity in the clearance plot. K value naturally decreased as ICG dose was increased. And it was also confirmed that anesthesia with thiopental sodium with or without laparotomy had no influence on K values with this dose.

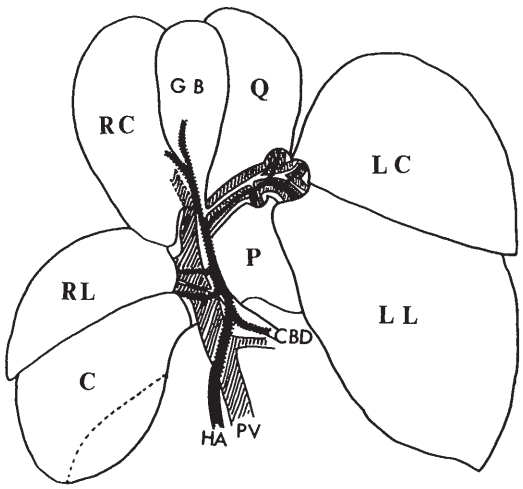

Fig. 3. Topography of the dog liver.
C, caudate lobe; RL, right lateral; RC, right central; Q, quadrate; LC, left central; LL, left lateral; P, papillary.

hepatectomy was carried out as described. Care was taken not to manipulate the hepatic lobes themselves to avoid any influence on their function. Eleven to 13% functional hepatectomies were made by ligation of hepatic artery, portal vein and bile duct of the caudate lobe; 17 to 30% hepatectomy, of caudate and right lateral lobes; 67 to 89% hepatectomy, of right central, quadrate, papillary, left central and left lateral lobes; 78 to 90% hepatectomy, of right central, quadrate, papillary, left central, left lateral and caudate lobes. After the abdomen was closed, an ICG study was conducted and then the dogs were sacrificed. The percentage of functional hepatectomy was retrospectively determined by weighing the liver lobes separately after exsanguination of the organ. According to the percentage of functional hepatectomy, the dogs were divided into the following six groups. Group A consisted of 3 dogs subjected to less than 20% functional hepatectomy; group B (25–30% hepatectomy), 5 dogs; group C (65–74% hepatectomy), 6 dogs; group D (75–79% hepatectomy), 5 dogs; group E (80–84% hepatectomy), 4 dogs; group F (85–90% hepatectomy), 5 dogs; group G (total functional hepatectomy), one dog.

STUDIES IN CCl_4 INTOXICATED DOGS: Twenty eight dogs were used. Control study of K of ICG was made following intravenous anesthesia, and then carbon tetrachloride (CCl_4, special grade. Katayama Chemical) was injected subcutaneously in varying doses. After 24 hours, K of ICG was studied, and the serum glutamic oxaloacetic transaminase (GOT) and the serum glutamic pyruvic transaminase (GPT) were measured by Hitachi M400 autoanalyser, and liver biopsies were obtained for microscopic histological examinations. According to the dose level of CCl_4, 28 dogs were divided into six groups. Group I (9 dogs) received no CCl_4; group II (2 dogs), 0.25ml CCl_4/kg body weight; group III (4 dogs), 0.5ml CCl_4/kg; group IV (4 dogs), 1.0ml CCl_4/kg; group V (4 dogs), 1.5ml CCl_4/kg; group VI (5 dogs), 2.0ml CCl_4/kg.

RESULTS

Results of the studies in dogs with functional hepatectomy are summarized in Table 1. Mean control K value was 0.100 ± 0.019 min.$^{-1}$ (mean ± S.D.) and K value and percentage of functional hepatectomy were in a negative correlation ($r = -0.88$, $p < 0.001$) (Fig. 4). For the purpose of expressing the function of the liver cell mass more precisely, a new concept of "changing rate of K" was employed, and this is defined by the following formula:[6] changing rate of $K = \frac{K \text{ after functional hepatectomy} - K \text{ (control)}}{K \text{ (control)}} \times 100$. Changing rate in 14% functional hepatectomy (Group A) was −3%; in 27% hepatectomy (Group B), −22%; in 72% hepatectomy (Group C), −44%; in 77% hepatectomy (Group D), −55%; in 82% hepatectomy (Group E), −64%; in 87% hepatectomy (Group F), −72%. Deviation of changing rate from normal (± 0%) was minimal in less than 20% functional hepatectomy, and changing rate increased its negativeness almost linearly as the percentage of functional hepatectomy increased up to 70%, but the curve showed a sharp drop beyond 70% (Fig. 5). K in total functional hepatectomy was measured in only one dog.

Table 1. K value and changing rate of K in each group of functional hepatectomy

	Percentage of (functional hepatectomy)	K after functional hepatectomy(min.$^{-1}$)	Changing rate of K (%)
Group A	(13.7 ± 3.1)	0.084 ± 0.009	−3.3 ± 1.5
Group B	(27.0 ± 2.9)	0.085 ± 0.020	−21.6 ± 6.4
Group C	(71.5 ± 2.9)	0.049 ± 0.003	−44.0 ± 6.3
Group D	(76.6 ± 1.1)	0.048 ± 0.007	−55.4 ± 14.3
Group E	(82.3 ± 1.3)	0.039 ± 0.005	−64.0 ± 9.3
Group F	(86.8 ± 2.5)	0.026 ± 0.002	−72.4 ± 3.3
Group G	(100)	0.013	−88.0

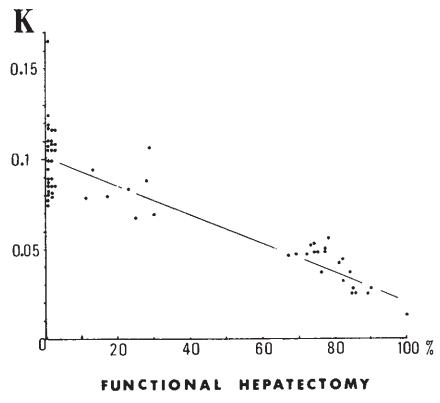

Fig. 4

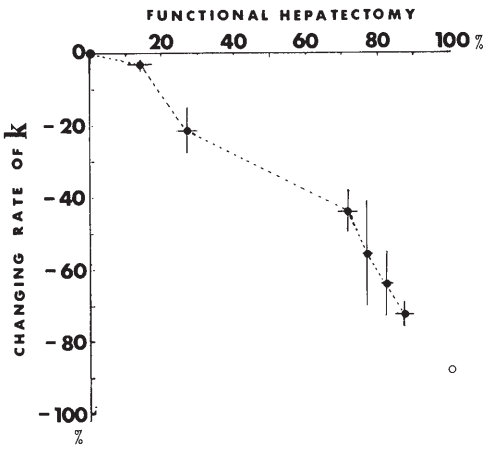

Fig. 5

Fig. 4. Correlation between percentage of functional hepatectomy and fractional clearance (K) of ICG; $r = -0.88$, $n = 58$, $p < 0.001$; regression line $y = -0.0008x + 0.1015$.

Fig. 5. Relationship between the percentage of functional hepatectomy and changing rate of K.

Table 2. K value, changing rate of K and serum GOT and GPT levels in each group of CCl_4 intoxication

	K 24 hrs. after CCl_4 injection	Changing rate of K (%)	GOT mean (range)	GPT mean (range)
Group I	(0.101 ± 0.030)*		25 (15– 43)	27 (18– 43)
Group II	0.140 ± 0.001	+22.9 ± 1.8	54 (35– 73)	52 (40– 64)
Group III	0.112 ± 0.008	+20.3 ± 15.9	64 (25– 83)	50 (34– 81)
Group IV	0.085 ± 0.031	−10.8 ± 19.2	152 (30– 352)	227 (39– 600)
Group V	0.085 ± 0.024	−11.5 ± 21.0	318 (68– 900)	760 (94–1990)
Group VI	0.044 ± 0.018	−52.6 ± 12.2	1551 (474–2750)	2670 (1720–4050)

* No administration of CCl_4.

The results of the studies in dogs with liver damage by CCl_4 are summarized in Table 2. Mean control K value in these dogs was 0.101 ± 0.030 min.$^{-1}$ (mean ± S.D.), serum GOT, 25 units (range 15–43), and serum GPT, 27 units (range 18–43) (Group I). K values 24 hours after injection of smaller doses of CCl_4 (groups II and III, the figures being 0.140min.$^{-1}$ and 0.112 min.$^{-1}$ respectively) were higher than control (Group I). K values 24 hours after injection of larger doses of CCl_4 were very low (0.044 min.$^{-1}$). However, all changing rates were positive in group II (range +22 to +24%) as well as in group III (range +7 to +43%). It may have indicated that hepatic function was better than control in dogs given smaller doses of CCl_4. Changing rates were between −39 to +4% in group IV and between −26 to +8% in group V. And all changing rates (range −33 to −65%) were negative in group VI (Fig. 6). Serum GOT and GPT levels were within normal range or only modestly increased in dogs with positive changing rates. And serum GOT and GPT were markedly increased (GOT, over 325 μ. and GPT, over 600 μ.) in dogs with changing rate below −26%. In group VI serum GOT and GPT levels were particularly high, i.e. mean GOT was 1551 μ. and mean GPT was 2670 μ. On microscopic examination, the liver of dogs with positive or slightly negative changing rates showed vacuolized cytoplasm in the centrolobular area but the hepatic cells were preserved normally in the periportal zone and there was no destruction of lobular architecture (Fig. 7). Dogs with highly negative changing rates showed extensive centrolobular necrosis with extensive bleeding and normal hepatic cells were scarcely seen in group VI (Fig. 8).

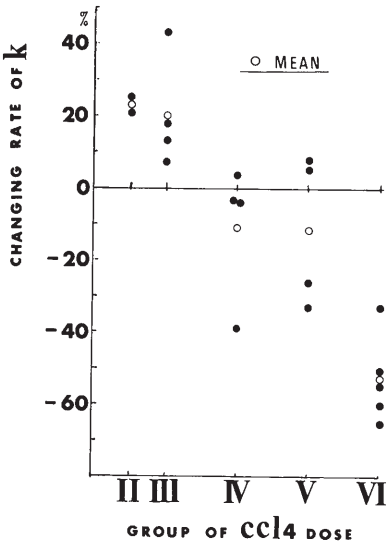

Fig. 6. Changing rates 24 hours after administration of CCl_4 in various doses.

DISCUSSION

ICG given intravenously is rapidly bound to plasma protein and as rapidly removed by the liver.[3~5] In contrast to BSP,[7~8] over 95% of administered ICG is recovered in the bile,[4] no significant difference in dye concentration is observed between general peripheral arterial and venous blood,[3,5] ICG is transferred less readily into the hepatic lymph after biliary obstruction,[9] urine collected during a constant infusion of ICG

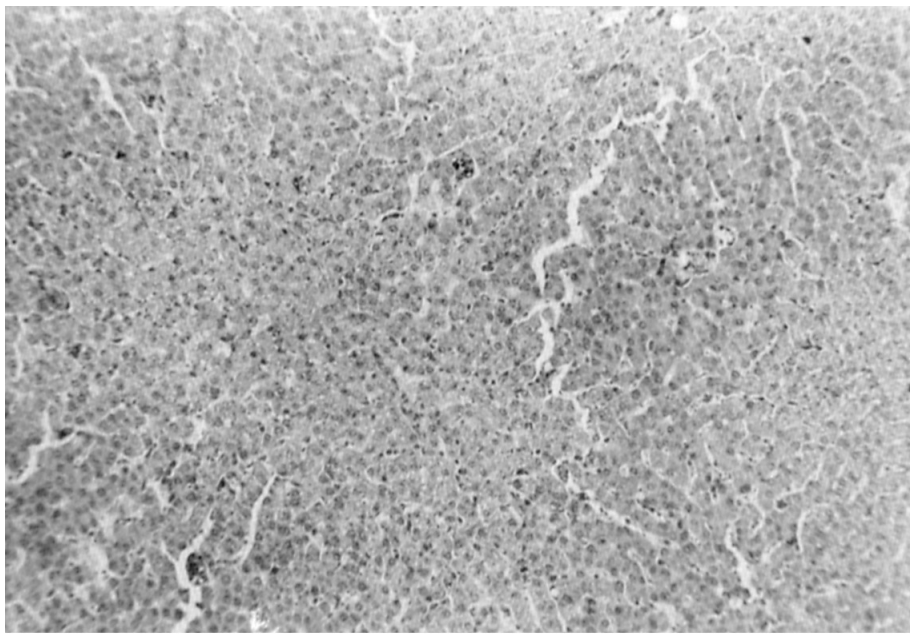

Fig. 7. Photomicrography of the liver acutely intoxicated with CCl_4 (Group III). Twenty-four hours after CCl_4 injection. K, 0.112 $min.^{-1}$; changing rate, +18%; GOT, 82 μ; GPT, 47μ. Vacuolization of cytoplasm is seen. Hematoxylin-eosin stain. × 100.

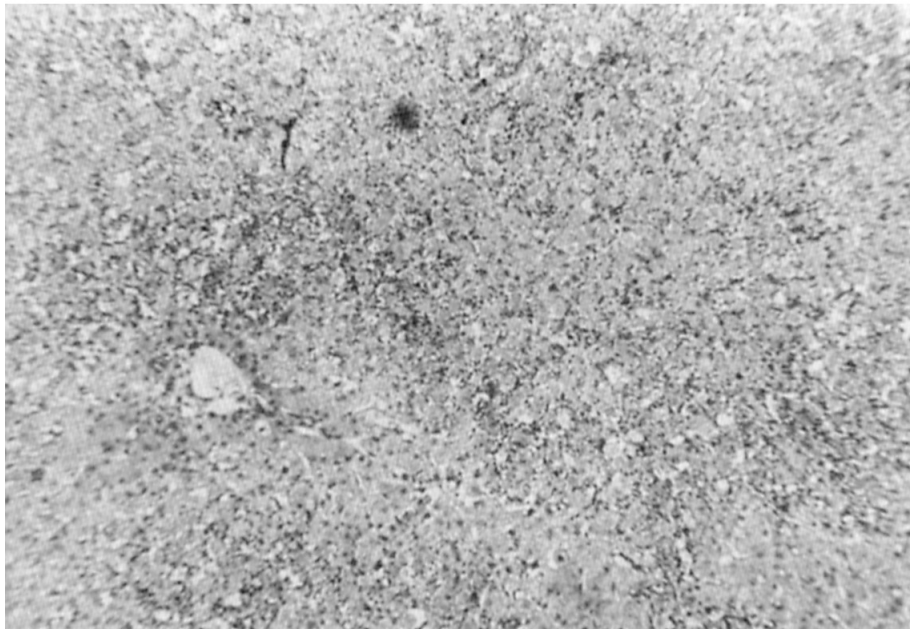

Fig. 8. Photomicrography of the liver acutely intoxicated with CCl_4 (Group VI). Twenty-four hours after CCl_4 injection. K, 0.029 $min.^{-1}$; changing rate, –60%; GOT, 2750 μ.; GPT, 4050 μ. Necrosis accompanied by bleeding is extensively seen, and the normal lobular architecture is scarcely seen. Hematoxylin-eosin stain. × 100.

contains no appreciable amount of dye,[3~5] and no entero-hepatic circulation has been confirmed.[5,9] Therefore, ICG is considered to be more useful than BSP in the evaulation of hepatic function and hepatic blood flow.[3,5,9~11] There have been many re-reports[3,5,9~10,12~15] that the clearance of ICG could be a valuable parameter in the differential diagnosis of various liver diseases. The clearance method provides an excellent and relatively simple means of assessing liver function.[16] The theoretic formulation of the concept of hepatic clearance adapting the method and concept of renal clearance was first explicitly made by Lewis.[17] Fauvert[18] suggested that the clearance could be in pathologic conditions of the liver, a simple method of evaluating the functional significance of impairment of the liver cells and the reduction of the hepatic blood flow, and that the clearance was a universal and fairly quantitative evaluation of the functional mass of the liver by exemplifying the changes in BSP clearance during the regeneration following partial hepatectomy in the rat. According to Rikkers *et al.*,[19] K of ICG decreases as the dose of the dye is increased, as was testified by the preliminary experiment in the present study. For clinical purposes, 0.25 to 1.0mg/kg body weight of ICG has been used.[3,5,9,11] Leevy *et al.*[12] reported that K in smaller dose of ICG was useful in evaluating hepatic function in patients with moderate to severe liver disease, and the larger dose was needed to detect mild hepatic disease, postulating that the working factor in removal of small dose might be liver blood flow rather than hepatocellular function, introducing the concept of the critical dose. Rikkers[19~20] and Moody[21] recommended that estimation of maximal removal rate of ICG provided a relatively reliable method of quantitating hepatic mass since the removal rate in the usual doses could not assess hepatic functional reserve adequately. On the other hand, Suzuki[6] reported that K of ICG was more dependent on functional liver cell mass than on liver blood flow while K of Au 198 colloid was definitely dependent on liver blood flow. Nishiwaki[22] and Ando[23] were also of the opinion that, since K of ICG decreased in spite of increased hepatic blood flow in dogs with liver damage, K of ICG was useful in assessing hepatic function.

Less than 20% functional hepatectomy induced only a minimal decrease in K of ICG, presumably hepatic function being adequately compensated by the hepatic functional reserve. On the contrary, more than 70% functional hepatectomy caused a significant decrease in K of ICG (Fig. 5). These results may indicate that K of ICG has a good correlation with the functional hepatic cell mass. If these results could be applied to clinical material, K value of 0.1 $min.^{-1}$ (normal value of K, 0.195 ± 0.037) would correspond to the hepatic function after 73% functional hepatectomy in dogs. It is reasonable, therefore, to set out the K value of 0.1 $min.^{-1}$ or more as an index of operability in cirrhotic portal hypertension (Fig. 2) and to limit the extent of hepatectomy within 70% in general hepatectomy cases.

CCl_4 produces histochemically detectable changes in the centrolobular hepatic parenchymal cells.[24] It has been simply considered that administration of CCl_4 decreases K of ICG.[9,22] The present study indicated, however, that changing rate of K of ICG increased more than normal control, when the dose of CCl_4 was small, despite the induction of vacuolization of centrolobular hepatic cells as well as elevation of serum GOT and GPT levels. This fact may be explained by the hyperfunction of the intact hepatocyte in ICG uptake. Large amount of CCl_4 produced extensive hepatocyte necrosis and remarkable elevation of serum GOT and GPT, and K values decreased in parallel with the degree of reduction of functioning hepatic cell mass.

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