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SIGNIFICANT ROLE OF INTRAHEPATIC SHUNT IN HEPATIC FUNCTION TEST WITH THE USE OF DYE IN CIRRHOSIS

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ABSTRACT

Using hepatic vein catheterization, fractional clearance (K) and hepatic extraction rate (ER) of Indocyanine Green (ICG) and radioisotope particles (RI) were studied in cirrhotic portal hypertension (24 postnecrotic cirrhotics and 25 alcoholic cirrhotics).

ICG given intravenously is cleared from blood only by hepatocytes, whereas RI are uptaken by intrahepatic (Kupffer cells) and extrahepatic reticuloendothelial (RE) cells.

Since there were significant positive correlations between K-ICG and K-RI in postnecrotic cirrhosis ($r = 0.588$, $p < 0.01$) and in alcoholic cirrhosis ($r = 0.620$, $p < 0.01$), and between ER-ICG and ER-RI in postnecrotic cirrhosis ($r = 0.508$, $p < 0.02$) and in alcoholic cirrhosis ($r = 0.558$, $p < 0.01$), the most reasonable explanation of these correlations was that significant amount of ICG and RI given intravenously bypassed both Kupffer cells and hepatocytes in the patients with portal hypertenaion due to cirrhosis.

Therefore, liver function test with the use of dye, such as ICG, is influenced significantly by intrahepatic shunt bypassing hepatocytes in cirrhosis.

INTRODUCTION

Using hepatic vein catheterization, various hemodynamic and metabolic evaluation of liver disease are possible. Hydrostatically wedged hepatic vein pressure (WHVP) reflects postsinusoidal pressure which is important to decide the location of obstruction or resistance against portal blood flow in the liver.[1-4] In alcoholic cirrhosis, WHVP has been shown to be equal to portal vein pressure.[4]

By administration of dye, such as Indocyanine Green (ICG) and Bromsulphalein (BSP), fractional clearance (K) and hepatic extraction rate (ER) of dye are obtained, which has been accepted as adequate estimation of liver function.[5] Also hepatic blood flow could be calculated from Fick principle using ICG or radioisotope colloid (RI) such as Au^{198}(Au) and I^{131}-RISA.[6,7] ICG is cleared from blood only by hepatocytes,[8] while RI are uptaken both by intrahepatic (kupffer cells) and extrahepatic reticuloendothelial (RE) cells.[9]

The purpose of the study was to try to evaluate each function of hepatocytes and Kupffer cells independently and to evaluate influencing factors on dye kinetics with the use of ICG and RI in subjects with cirrhotic portal hypertension.

MATERIALS AND METHOD

The patients studied were divided into following two groups; Group A: Twenty four

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postnecrotic cirrhotics (Group III in Imanaga's classification)[1], and Group B*: Twenty five alcoholic cirrhotics.[4]

All patients studied had esophageal varices which were demonstrated by upper gastrointestinal series and/or esophagoscopic examination. Degree and type of liver disease were defined histologically by surgical wedge biopsy in Group A and by percutaneous aspiration biopsy with Menghini needle in Group B.[10] Hepatic vein catheterization was performed under local anesthesia in fasting state via antecubital vein. WHVP were obtained in at least two different hepatic veins and pressure was expressed in mmH_2O above right atrial pressure (RAP) in Group A, and above inferior vena cava pressure (IVCP) in Group B.[4] After WHVP was obtained, the tip of the catheter was kept in free position in hepatic vein. Then 0.5 mg per kg of ICG[11-13] and 50 microCi of Au[14] in Group A, or 2 mCi of technetium-99m sulfur (Tc)[9,15] in Group B were mixed in the syringe with 0.2 ml of albumin or a few ml of patient's own blood, which were given intravenously. Blood samples were drawn simultaneously from hepatic vein through the hepatic vein catheter and peripheral vein every two or three minutes for twelve minutes, and were analyzed for ICG content and radioactivity. ICG content and radioactivity of each samples were plotted on semilogarithmic graph with time as the abscissa. Each of ICG contents and radioactivities in blood decrease exponentially which is expressed as following formula: $C_t = C_0 e^{-Kt}$, where t is the time in minutes, C_0 is content or radioactivity extrapolated back to zero time, C_t is content or radioactivity at given time t, and K is constant or fractional clearance. When these data are plotted on the semilogarthmic graph, exponential curve is expressed as linear line. K is calculated form the formula: $\ln 2/T^{1/2}$, where ln 2 is the natural logarithm of 2 or 0.693, and $T^{1/2}$ is the time in minutes required for the concentration of ICG or radioactivity to diminish by 50 per cent.[7] ER was calculated from the formula: $\frac{P_0 - H_0}{P_0}$, where P_0 is ICG content or radioactivity in peripheral blood samples extrapolated back to zero time, and H_0 is that in hepatic vein blood samples extrapolated back to zero time.[7]

For comparison of characteristics of Group A and B, thrombotest (Owren method) which has been thought to reflect hepatocyte function, serum globulin, WHVP, K-ICG, ER-ICG, K-RI, and ER-RI were evaluated.

RESULTS

Results were summarized in Table 1 (Group A) and Table 2 (Group B). WHVP was 244 ± 61 mmH_2O (± SD) above RAP in Group A and 208 ± 61 mmH_2O above IVCP in Group B (Figure 1). Thrombotest was 56 ± 19% (± SD) in Group A and 58 ± 22% in Group B (Figure 2). Serum globulin was 3.3 ± 0.2gm/dl (± SD) in Group A and 4.3 ± 0.94gm/dl in Group B (Figure 2). K-ICG was 0.106 ± 0.042 (± SD) in Group A and 0.051 ± 0.036 in Group B (Figure 3).

ER-ICG was 0.382 ± 0.216 (± SD) in Group A and 0.188 ± 0.151 in Group B (Figure 3). K-Au was 0.115 ± 0.043 (± SD) in Group A and K-Tc was 0.117 ± 0.038 in Group B (Figure 4). ER-Au was 0.418 ± 0.167 (± SD) in Group A and ER-Tc was 0.305 ± 0.147 in Group B (Figure 4).

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* Group B were studied by the author at John Wesley County Hospital, Los Angeles, California, using the same technique.

Table 1. Results of Group A.
WHVP; wedged hepatic vein pressure in mmH_2O above right atrial pressure

#	WHVP	ICG		Au		Glob.	Proth.
		ER	K	ER	K		
1.	260	0.070	0.120	0.390	0.097	2.8	–
2.	190	0.039	0.097	0.160	0.069	3.8	–
3.	190	0.348	0.125	0.437	0.156	3.9	–
4.	180	0.590	0.099	0.780	0.192	3.5	48
5.	225	0.577	0.200	0.245	0.210	3.5	–
6.	220	0.540	0.062	0.524	0.082	3.5	–
7.	380	0.157	–	0.185	0.086	3.5	34
8.	330	0.321	0.091	0.435	0.116	3.1	–
9.	250	0.210	0.077	0.520	0.080	2.1	–
10.	345	0.307	0.073	0.223	0.083	4.2	–
11.	230	0.283	0.100	0.530	0.079	2.7	–
12.	195	0.156	0.075	0.250	0.101	2.8	80
13.	300	0.200	0.079	0.600	0.158	3.9	54
14.	240	0.510	0.063	0.370	0.069	2.9	55
15.	150	–	0.082	0.500	0.119	2.4	–
16.	200	0.430	0.161	0.520	–	2.7	–
17.	330	0.690	0.130	0.510	0.084	4.9	–
18.	250	0.370	0.110	0.590	0.042	4.3	43
19.	220	0.400	0.065	0.440	0.126	3.0	100
20.	180	0.690	0.173	0.380	0.100	2.5	72
21.	275	0.720	0.192	0.608	0.169	3.3	–
22.	150	0.450	0.128	0.440	0.150	2.9	40
23.	265	0.730	0.115	0.460	0.115	2.8	37
24.	310	0.025	0.023	0.040	0.002	5.2	56

ER; Hepatic extraction rate.
K; Fractional clearance.
Glob.; Serum globulin (gm/dl).
Proth.; Thrombo-test (Owren method).

Table 2. Results of Group B.
WHVP; Wedged hepatic vein pressure in mmH_2O above inferior vena cava pressure

#	WHVP	ICG		Tc		Glob.	Proth.
		ER	K	ER	K		
1.	169	0.220	0.074	0.460	0.165	–	–
2.	260	0.200	0.007	0.100	0.065	4.3	43
3.	247	0.070	0.030	0.270	0.107	3.6	38
4.	156	0.090	0.024	0.120	0.112	3.8	23
5.	245	0.200	0.043	0.200	0.060	4.7	70
6.	196	0.210	0.022	0.160	0.043	–	36
7.	247	0.226	0.124	0.244	0.182	4.2	80
8.	230	0.077	0.037	0.111	0.103	2.8	72
9.	247	0.170	0.042	0.115	0.107	–	–
10.	338	0.143	0.043	0.321	0.077	6.7	–
11.	260	0.690	0.167	0.596	0.173	3.1	100
12.	195	0.250	0.060	0.490	0.116	–	–
13.	182	0.099	0.028	0.192	0.099	4.3	52
14.	247	0.134	0.058	0.322	0.135	4.7	92
15.	130	0.336	0.068	0.344	0.095	4.7	52
16.	156	0.053	0.038	0.382	0.128	–	44
17.	169	0.123	0.035	0.229	0.082	5.7	–
18.	156	0.022	0.017	0.245	0.099	4.0	–
19.	312	0.041	0.023	0.500	0.135	–	–
20.	169	0.093	0.043	0.195	0.116	–	–
21.	169	0.071	0.025	0.217	0.126	–	–
22.	117	0.454	0.064	0.543	0.116	–	–
23.	91	0.389	0.107	0.517	0.173	–	–
24.	195	0.095	0.041	0.440	0.201	–	–
25.	–	0.240	0.053	0.324	0.112	–	–

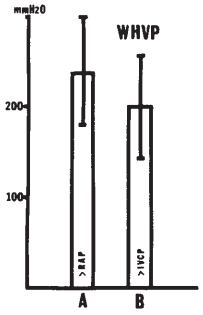


Fig. 1

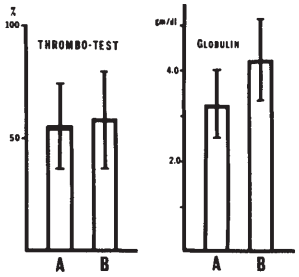


Fig. 2

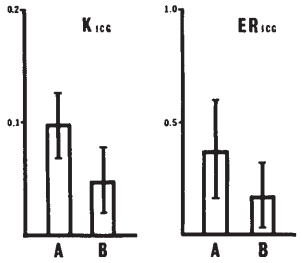


Fig. 3

Fig. 1. Wedged hepatic vein pressure (WHVP). Pressure was expressed in mmH_2O above right atrial pressure (RAP) in Group A and above inferior vena cava pressure (IVCP) in Group B. (± SD)
Fig. 2. Thrombo-test (Owren method, normal; more than 80%) and serum globulin (gm/dl). (± SD)
Fig. 3. Fractional clearance (K-ICG) and hepatic extraction rate (ER-ICG) of Indocyanine Green (ICG) in Group A and Group B. (± SD)

There was significant positive correlation between K-ICG and K-Au in Group A ($r = 0.588$, $p < 0.01$) (Figure 5). Between ER-ICG and ER-Au, significant positive correlation was noted in Group A ($r = 0.508$, $p < 0.02$) (Figure 6). Also in Group B, there were significant positive correlations between K-ICG and K-Tc ($r = 0.620$, $p < 0.02$) (Figure 7), and between ER-ICG and ER-Tc ($r = 0.558$, $p < 0.01$) (Figure 8).

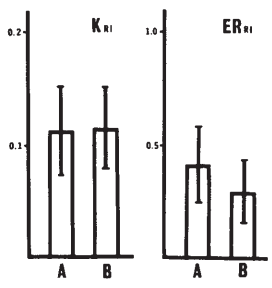
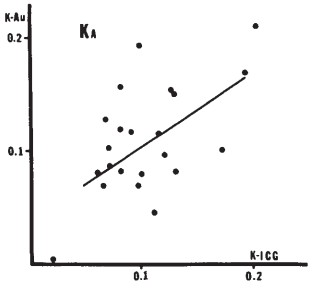


Fig. 4



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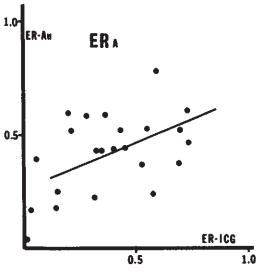

Fig. 6

Fig. 4. Fractional clearance (K-Au) and hepatic extraction rate (ER-Au) of ^{198}Au in Group A, and of Tc^{99m} sulfur (K-Tc and ER-Tc) in Group B. (± SD)
Fig. 5. Correlation between K-ICG and K-Au in Group A. $r = 0.588$, $p < 0.01$ Regression line: $y = 0.667x + 0.040$.
Fig. 6. Correlation between ER-ICG and ER-Au in Group A. $r = 0.508$, $p < 0.02$ Regression line: $y = 0.390x + 0.268$.

ER-Au were correlated poorly with serum globulin ($r = -0.264$), and ER-ICG were correlated poorly with serum globulin ($r = -0.112$) in Group A. Also serum globulin was correlated poorly with ER-Tc ($r = 0.019$) and ER-ICG ($r = -0.194$) in Group B.

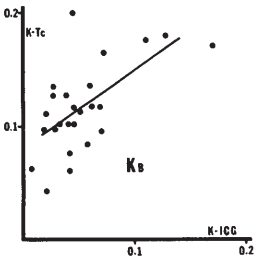

Fig. 7

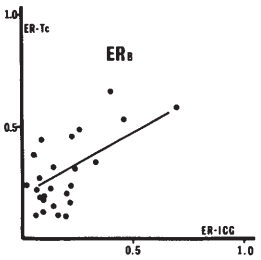

Fig. 8

Fig. 7. Correlation between K-ICG and K-Tc in Group B.
r = 0.620, $p < 0.01$ Regression line: $y = 0.672x + 0.083$.
Fig. 8. Correlation between ER-ICG and ER-Tc in Group B.
r = 0.558, $p < 0.01$ Regression line: $y = 0.554x + 0.201$.

DISCUSSION

Both postnecrotic (Group A) and alcoholic cirrhosis (Group B) showed almost identical hepatic hemodynamics and have been grouped into intrahepatic postsinusoidal portal hypertension.[1,3,4] However, outcome of portacaval anastomosis (PCA) was different from each other. The patients with alcoholic cirrhosis seem to tolerate PCA better than the patients with postnecrotic cirrhosis.[16,17] Reasons of this discrepancy between these two groups have not been settled yet.

Since RAP was lower than IVCP approximately 30 mmH_2O or so to our experience at hepatic vein catheterization, subjects of Group A and Group B in this study had almost the same degree of portal hypertension.[4]

ICG given intravenously bounds with serum albumin[8] and alpha-l-lipoprotein[18,19] rapidly, which is cleared from blood solely by hepatocytes without appreciable enterohepatic circulation.[13] Based on this concept, ICG has been used for evaluation of hepatocellular function.[11-13] K-ICG has been evaluated extensively in various liver diseases, but ER-ICG has been evaluated limitedly because of necessity to employ hepatic vein catheterization. RI, such as Au and Tc, are uptaken by RE cells located in the liver, spleen and bone marrow. Blood disappearance half time of Tc is 2.5 to 3.5 minutes (K = 0.255 – 0.277), and that of Au is approximately 4.5 minutes (K = 0.154) in normal subjects.[20] This difference of K between Tc and Au must be due to the fact that Tc is larger particle than Au, and larger particles have higher chance to be trapped by RE cells than smaller particles.[21-22] Each materials have been used for liver scan. In normal subjects, uptake of these RI is observed mainly in the liver and spleen, but bone marrow uptake is not visualized at liver scan. To the contrary, in patients with chronic liver disease, such as chronic active hepatites and cirrhosis, mottling appearance of the liver image and increased uptake by the spleen and bone marrow are remarkable at liver scan,[23-25] which has been explained by hyperactive or hypertrophic RE cells in chronic liver diseases.[26] In this study, there were significant positive correlations between K-ICG and K-RI in Group A ($r = 0.588$, $p < 0.01$) and in Group B ($r = 0.620$, $p < 0.01$) (Figure 5, 7). Also there were significant positive correlations between ER-ICG and ER-RI in Group A ($r = 0.508$, $p < 0.02$) and in Group B ($r = 0.558$, $p < 0.01$) (Figure 6, 8).

Thus, almost identical extraction and disappearance of ICG and RI were observed in

Group A and in Group B. There are three possible explanations of these significant positive correlations: (1) Total number of hepatocytes and Kupffer cells, i.e. functional mass of the liver, decreased as the degree of liver cirrhosis was severer because of collapse of normal hepatic parenchyma and formation of non-functioning scar tissue, (2) Both hepatocyte and Kupffer cell function were depressed as the degree of liver cirrhosis, (3) Significant amount of ICG and RI which were given intravenously bypassed both hepatocytes and Kupffer cells through pathologically created portal-systematic shunt.

In the area of preserved parenchyma or regenerated nodules, Kupffer cells were rather hyperactive and increased in number, whereas collapsed area which was consisted of fibrotic tissue, proliferated capillaries and bile ductules contained almost no Kupffer cells and hepatocytes. Total number of each cells were difficult to assess in each patient from this study. In congenital hepatic fibrosis which causes severe portal hypertension, liver size itself is not smaller than normal, but wide and dense fibrotic tissue containing proliferated bile ductules occupies significant volume of the liver, and still shows normal or near normal K and ER of ICG and RI. Probably positive correlation between K and ER of ICG and RI in both Group A and Group B were not directly depend on total number of Kupffer cells and hepatocytes.

Kupffer cell function as phagocytosis seemed to be hyperactive in microscopic examination of liver tissue in both postnecrotic cirrhosis and alcoholic cirrhosis. However, hepatocyte function per se will be depressed because there were scattered forcal necrosis, fatty change and abnormaly deformed hepatocytes. It is not primary reason to explain these significant positive correlation between K and ER of ICG and RI in both Group that Uptake of RI by Kupffer cells decreased because of hypofunction of Kupffer cells.

There are two kind of portal-systemic shunt in cirrhosis, one is extrahepatic and the other is intrahepatic shunt. Extrahepatic shunt per se does not influence significantly on bypass phenomenon of hepatocytes and Kupffer cells, since shunted blood flow through extrahepatic shunt was only 57 ml/min/m^3 in mean value, whereas that through intrahepatic shunt was 240 ml/min/m^3.[27] Intrahepatic shunt is created pathologicaly in collapsed and fibrotic tissue after destruction of liver parenchyma by progressive changes in the hepatic perisinusoidal tissue space of Disse leading to development of a basement membrane and capillarization of sinusoids. The transformation of an open circulation to a close one decreases the effectiveness of hepatic circulation and aggravates hepatocellular insufficiency.[28] Presumably, among these three explanations, significant amount of RI and ICG bypassed hepatocytes and Kupffer cells through intrahepatic shunt which was created pathologicaly in fibrous tissue after destruction of hepatic parenchyma, and dysfunction of both hepatocytes and Kupffer cells, size of the liver or total number of hepatocytes and Kupffer cells might give less influence on existence of positive correlation between K and ER of RI in both Group A and Group B.

Mean thrombotest in Group A and Group B were nearly equal, suggesting almost the same degree of hepatocyte function (Figure 2), but mean K-ICG and ER-ICG in Group A were better than those of Group B (Figure 3). Presumably Group B should have higher degree of intrahepatic shunt than Group A. Alcoholic cirrhosis is micronodular type, whereas postnecrotic cirrhosis is macronodular type. Nakamura demonstrated that micronodular type of cirrhosis had higher degree of intrahepatic shunt than macronodular cirrhosis.[27] In alcoholic cirrhosis, ER and K or RI improved after PCA because of shutdown of intrahepatic shunt by end-to-side PCA.[7] The reason why alcoholic cirrhosis could tolerate PCA better than postnecrotic cirrhosis might be explained partially from the fact

that PCA in alcoholic cirrhosis obliterates intrahepatic shunt which is more predominant than in postnecrotic cirrhosis, and produces better perfusion of sinusoid contacting with hepatocytes and Kupffer cells because of compensative improvement of hepatic arterial blood flow after PCA.

From described above, intrahepatic shunt developed remarkably in both Group A and Group B, and liver function test with the use of ICG reflects not only hepatocyte function itself and functional volume of the liver, but also degree of intrahepatic shunt significantly in cirrhosis.

It has been well appreciated that serum globulin elevates in cirrhotics.[29] This has been explained as following; enterogenic antigens absorbed in portal blood from the gut cannot be trapped and extracted by Kupffer cells because of intre- or extrahepatic shunt bypassing Kupffer cells, and chronically stimulate RE cells resulting in production of much globulin.[30, 31] However, there were poor correlations between serum globulin and ER-RI and ER-ICG in Group A and Group B, suggesting that hyperglobulinemia in cirrhotics was not primarily due to intrahepatic shunt.

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