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–Original Article–

THE CONSTITUTIONAL CONJUGATED HYPERBILIRUBINEMIA (Dubin-Johnson Syndrome and Rotor's Type of Hyperbilirubinemia) New Definitions Based on Studies of Transport with Indocyanine Green and Bromsulfophthalein

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Summary

Plasma clearance studies with Indocyanine Green (ICG) and Bromsulfophthalein (BSP) were performed on four patients with chronic conjugated hyperbilirubinemia with or without apparent brown pigments in the liver cells, and free from structural hepatic diseases.

An abnormal clearance pattern, characterized by reduced hepatic ICG and BSP clearances, was observed in one case with Rotor's type of hyperbilirubinemia (R). In the other three cases of Dubin-Johnson syndrome (DJ), an abnormal disappearance curve with a secondary rise of serum BSP 60 and 120 minutes after intravenous administration, was observed, although the hepatic disappearance rates of both ICG and BSP were normal. A two-compartmental analysis revealed that the depressed hepatic dye clearance in R might be due to defects both in the uptake and in the excretion of dye by the liver, and there seems to be an excretory defect of the dye in DJ.

The studies on the transport maximum (Tm) and the relative storage capacity (S) of ICG demonstrated the reduction of Tm and S in R, while decreased Tm and normal S in DJ. These results suggest that the current diagnostic criteria for DJ and R would be based upon the dye transport distinction in patients with the same basic hyperbilirubinemia.

Dubin-Johnson Syndrome (DJ) and Rotor's Syndrome (R) are characterized by the presence of chronic, mediumgrade conjugated hyperbilirubinemia in otherwise normal subjects.

The diagnosis of these two syndromes is usually restricted to patients with normally functioning liver except for a dye excretory dysfunction, and those who showed no evidence of overt hemolysis.

The hyperbilirubinemia is presumed to be due to an abnormal hepatic excretion of conjugated bilirubin into the bile in case of DJ, and to an abnormal uptake of conjugated biluribin from the plasma by the liver in case of R.[1)]

Recent reports have demonstrated that the plasma BSP levels increased again 60

Key Words

1) Constitutional conjugated hyperbilirubinemia. 2) Dubin-Johnson syndrome. 3) Rotor's type of hyperbilirubinemia. 4) Indocyanine Green. 5) Bromsulfophthalein. 6) Transport maximum. 7) relative storage capacity. 8) *dye transport.*

and 120 minutes after intravenous administration,[2)3)] while the hepatic Tm of BSP decreased in DJ.[4)5)] As DJ is marked by the deposit of brown pigments in the liver cells, R is occasionally defined as a subtype of DJ in spite of the absence of the pigments.

This report describes the results of plasma clearance studies with BSP and ICG in one case of R, and in three cases of DJ in whom the absence of significant structural hepatic diseases was exemplified by appropriate examinations including liver biopsy.

Materials and Methods

Patients: Studies were performed on four patients at Juntendo University Hospital for the evaluation of chronic conjugated hyperbilirubinemia. The clinical findings and laboratory data are summarized in **Table I** and **II.** The following tests were normal in all cases: Serum glutamic oxaloacetic transaminase, serum glutamic pyruvic transaminase, cholesterol and its ester, serum protein concentration, serum protein electrophoresis, urinalysis except for bilirubinuria,

Table I. Clinical and laboratory data in four cases

Case No.	Age (yr) and Sex	Onset (yr)	Bilirubin (mg/ml) Direct	Bilirubin (mg/ml) Total	SGOT	Alkaline Phosphatase	Cholecystography OC	Cholecystography VC	Black Liver	Brown Pigment
1	20,M	20	3.9	6.4	8	13.5	NV	NV	+	±
2	26,M	16	3.3	6.0	20	5.8	NV		+	+
3	27,M	21	2.6	4.2	9	6.1	NV	GV	+	+
4	51,M	9	4.7	6.6	11	7.7	GV	NV	−	±
Upper limit of normal value in our lab.			0.4	0.6	40	9.9				

OC: Oral cholecystography
VC: Venous cholecystography
NV: not visualized
GV: good visualization

Table II. BSP clearance and ICG clearance, Tm and S, and parameters in four cases

Case No.	BSP 45 min Retent.	K	Secondary Rise	ICG K	Tm	S	λ21	λ12	λ02
1	6		+	0.185	0.67	144	0.2021	0.0057	0.0147
2	10		+	0.198	0.69	180	0.1921	0.0082	0.0323
3	5	0.099	+	0.198	0.82	625	0.2032	0.0033	0.0147
4	50	0.011	−	0.019	0.56	20	0.0317	0.0504	0.0341
Normal limit in our lab.									
upper	5	0.120	−	0.206	1.05	203	0.2294	0.0178	0.0845
lower	0	0.088		0.168	0.85	55	0.1948	0.0082	0.0435

K: Plasma diappearance rate
Tm: hepatic transport maximum
S: relative storage capacity

complete blood count, erythrocyte sedimentation rate, reticulocyte count, bleeding time, clotting time, prothrombin time, erythrocyte osmotic fragility, Coombs' test, CRP test, blood syphilis reaction and chest roentgenogram, and barium examination of the upper and the lower gastrointestinal tracts.

A liver biopsy was carried out on three of the four patients laparoscopically and percutaneously on a remaining case.

The serum BSP levels were measured by the method of Nishikaze.[8] The disappearance rate of BSP was calculated from the initial linear part of the curve after an intravenous administration of 5 mg of BSP per Kg body weight. BSP retention was estimated 45 minutes after an injection. The separation of BSP metabolites in serum was accomplished by ascending paper chromatography, using the method of Grodsky et al.[9]

The ICG concentration in serum was measured at the wave length of 805 mμ. The disappearance rate of ICG was computed from the initial linear part of the curve after the intravenous administration of 0.5 mg of ICG per Kg body weight. Because of the observation for the late clearance, the specimen was obtained for a longer period of time (60 to 120 minutes).

Tm and S for ICG were determined, using the modified method of Wheeler et al.,[4] established by Nambu.[21]

Results

Preliminary Data: Three patients(Case 1, Case 2 and Case 3) met the usual diagnostic criteria for DJ and one patient (Case 4) for R.[11][12]

Liver Biopsy: Three livers (Case 2, Case 3 and Case 4) were observed and biopsied laparoscopically. Two of them (Case 2 and Case 3) had the black coloration and another normal. In one patient (Case 1) the liver specimen, obtained percutaneously, showed black coloration. Except for the brown pigments in the liver cells, the histological examination under the light microscopy revealed no abnormalities of cellular morphology or lobular architecture, no fibrosis and no evidence of hepatitis were observed in any of the specimens examined. In cases with DJ moderate amounts of brown pigments were noted, while in R the amount of hepatic brown pigments was small.

BSP Test: The BSP retention in serum 45 minutes after single intravenous injection of 5 mg per Kg body weight was less than five per cent in subjects without hepatic disease. In cases of DJ it ranged from 5.0 to 10 per cent, and in R. was 50 per cent. The BSP disappearance rate of DJ (Case 3) was 0.099. In R its rate was 0.011, while in normal subjects it ranged between 0.088 and 0.120. The secondary rise of serum BSP lasting from 60 to 120 minutes after injection was observed in three cases of DJ. Measurement of BSP conjugates in the serum by paper chromatography demonstrated the predominant retention of conjugated BSP during the secondary rise in these cases. In R there was no secondary rise of serum BSP, and free BSP accounted for most of the chromatographic fraction of BSP.

ICG Test: The ICG disappearance rate in serum after a single injection of 0.5 mg per Kg body weight in subjects free of hepatic disease, ranged from 0.168 to 0.206. In DJ it was within normal range, and in R it decreased clearly. The determination of the Tm and the S values by the continuous ICG infusion technic showed decrease for Tm in all cases of DJ. Two cases indicated the

normal value and one case showed the elevated value for S in DJ. But, there was significant regression for Tm and S in R.

Kinetic Studies: From the serum ICG clearance curve the date for each test were fitted to a two-exponential function of the formula:[10)22)]

$Q(t)=K(A_1e^{-\alpha_1 t}A_2e^{-\alpha_2 t})$, where $A_1+A_2=1$, and K is a constant equal to the reciprocal of the initial volume of distribution of the injected ICG. Results based on two-compartmental analysis showed significant differences between DJ an R in λ_{21}, λ_{12}, λ_{02}, -the fractional transfer rates associated with hepatic ICG uptake, reflux to plasma, and elimination of ICG from the compartmental system. In DJ values of λ_{02} and λ_{12} were significantly reduced, while that of λ_{21} were almost normal. In R the value of λ_{21} and λ_{02} were apparently diminished, while the value of λ_{12} augmented.

Cholecystography: In R a normal gallbladder was observed on oral cholecystography but not in DJ. On intravenous cholecystography in three cases (Case 1, Case 2, and Case 4) gallbladder was not visualized. In Case 3 there was the normal visualization of gallbladder at 240 minutes after the intravenous administration of iodipamide.

Hepatic Scintiscanning: Hepatic scintiscan was observed after the intravenous administration of 5 μc per Kg body weight ^{131}I-labeled BSP in Case 3 and Case 5. In DJ the liver presented a fairly uniform distribution of radioactivity, although the gallbladder was not recognizable. In R the liver showed a generally reduced uptake, and the gallbladder was visualized faintly.

Discussion

In 1948 Rotor et al.[11)] first described the familial nonhemolytic jaundice with direct van den Bergh reaction. In 1954 the chronic idiopathic jaundice with unidentified pigments in the liver cells was documented by Dubin and Johnson.[12)] Both patients showed a moderate elevation of direct reacting bilirubin level in the serum. Liver histology was normal with or without lipofuscinlike pigment in the liver cells. Number of similar patients have been reported. Porush et al.[13)], on reviewing the ten cases R reported up to 1960, concluded that this syndrome is closely related to DJ. Arias[7)] found both R and DJ in the same family.

The major difference between R and DJ is that the former has no accumulation of pigments in liver cells, the normal visualization of the gallbladder on oral cholecystogram, and the greater average BSP retention. Despite these differences, there were several similarities. Wolf et al.[6)] reported two families with DJ who had normal or faint visualization of the gallbladder. The amount of deposited pigments varied according to the individuals. Blank et al.[14)] described three sisters with DJ. There were pigments in the liver cells in two of them. In case with DJ reported by Hunter et al.[15)] the liver biopsy demonstrated a complete mobilization of pigments from the hepatic cells during acute hepatitis, and reappearance in recovery. Two case of DJ accompanied by liver dysfunctions were reported by Hofstetter et al.[16)] They showed all the anatomical and histological features of the disease. Hepatic pigments may be found in patients with otherwise normal livers, and liver biopsies from patients with Gilbert disease.[17)] The distinction between the normal lipofuscin and the pigment found in DJ seems to be difficult.[18)19)] In our three cases of DJ and one case of R the livers had pigments in the cells. The pigments in R could not be distinguished from that

observed in DJ. Therefore, the pigment per se is most unlikely to be responsible for the absolutely essential clinical signs which differentiate these two syndromes each other.

Although it has been postulated that jaundice both in DJ and R is the result of an impaired hepatic excretory function.[20] BSP retention is occasionally found normal and is usually between 10 and 20% in DJ, and between 15 and 65% in R. According to Mandema et al.[2] the uptake of BSP by the liver during the initial 30 minutes after injection is considered to be normal. Our results of BSP loading test show no distinct defect in BSP uptake by the liver in DJ, but in R the defect is suspected. The disappearance rate of ICG represents normal or slight decrease in DJ. This decreased rate suggested blocking or competitive inhibition of ICG by the serum bilirubin. In our study the ICG disappearance rate was within normal range in case of DJ, and but in R it decreased apparently. The overall transfer of ICG from the plasma to the bile involves a number of processes, which are not clearly elucidated yet. It is supposed that ICG initially enters into the liver cells, and then the intracellular transport and active transport across the canalicular membrane into the biliary radicles occur. Each of these processes can be defined by a set of physicochemical constants. Our data indicate that the sequence of events including hepatic ICG uptake, intracellular transport and biliary excretion, can be considered as a function of the first order.

The ICG kinetic parameters in two-compartmental analysis from the disappearance curve of a single injection of ICG represents that in DJ the initial entry of ICG from the plasma into the liver cells is normal, the reflux of ICG from the liver cells to the plasma is reduced, and the transport of ICG across the canalicular barrier into the biliary radicles decreases. The ICG kinetic parameters in R show that the initial entry and the biliary transport are diminished, while the reflux to the plasma augmented.

Wheeler et al.,[4] using a constant infusion of BSP in three patients with DJ, calculated a normal storage capacity and observed a marked decrease in the maximal transfer of BSP to the bile. Schoenfield et al.[21] reported that a greater reflux of the conjugated BSP to the blood stream as comparable to the maximal transfer for BSP, which is less than 1 mg per minute in DJ. Our finding obtained by using ICG instead of BSP, indicated a normal or raised storage capacity and a fall in the maximal transfer of ICG to the bile in DJ, and distinct decrease in the storage capacity and in the maximal transfer in R.

Mandema et al.[2] proposed that a secondary rise of the conjugated BSP in the serum after a single injection was useful in the diagnosis of DJ. All of our cases with DJ have a secondary rise of serum BSP and this phenomena is characteristic in cases of DJ, and it has not been observed in other cases with liver dysfunctions or in R. In cases of R the appearance of the conjugated BSP is rare, in spite of the apparent retention of free BSP in the serum.

Normal visualization of the gallbladder on oral cholecystogram is one of the major signs which differentiate from DJ. In patients with R the gallbladder usually visualize normally, but in DJ pateints it failed to visualize. Wolf et al.[6] reported on two families with DJ, most of whom normal or faint visualization of the gallbladder. In DJ the gallbladder may be visualized on intravenous cholecystography at the advanced stage. In contrast, in R the intravenous cholecystogram less frequently gives the visualization of the gallbladder.

The scinti-scan by ^{131}I-labeled BSP showed poor hepatic uptake in R and suppressed excretion into the bile duct in DJ.

The foregoing suggests that there is a clear difference between these two syndromes as far the dye transport is concerned. In DJ there is mainly a defect in the late stage transport, such as the post-lysosomal transport, while the uptake and the storage capacity are normal. In R the defects are found in the early stage transport, such as the cellular uptake and the storage capacity is reduced but the excretion is slightly suppressed.

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