Asialoglycoprotein Receptor Function of Hepatocytes in Patients with Autoimmune Hepatitis

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Summary: To assess whether the hepatocyte asialoglycoprotein receptors are affected in patients with autoimmune hepatitis, the function of the hepatocyte asialoglycoprotein receptor was investigated in patients with autoimmune hepatitis or chronic hepatitis C. A new radionuclide liver imaging technique, Technetium-99m diethylenetriamine-pentaacetic acid-galactosyl human serum albumin, was applied to evaluate the function of the receptor. A Receptor Index (LHL 15) was calculated by dividing the radioactivity of the liver region-of-interest (ROI) by that of the liver plus heart ROI 15 min after radiolabeled ligand injection. Serum albumin, prothrombin time, hepaplastin test and plasma retention rate of indocyanine green at 15 min were not significantly different between patients in the autoimmune hepatitis group and chronic hepatitis C group. In addition, the Receptor Index of patients with autoimmune hepatitis, in whom the asialoglycoprotein receptor is a candidate target antigen for autoimmune response, was similar to that of patients with chronic hepatitis C. These results indicate that the hepatocyte receptor for asialoglycoproteins is not affected in patients with autoimmune hepatitis.

Key words: radionuclide imaging — autoimmune hepatitis — chronic hepatitis — asialoglycoproteins — hepatocyte

Introduction

The asialoglycoprotein receptor (ASGPR) in hepatocytes is a specific receptor for desialylated glycoproteins (Morell et al. 1968). This system is one of the best characterized receptor-ligand systems (Geuze et al. 1983; Schwartz et

al. 1983; Harada et al. 1992). After the internalization of ASGPs by the ASGPR, such ligands are directed to lysosomes, while the receptor recycles to the plasma membrane.

A new radionuclide liver imaging method using the ASGP receptor system was developed (Vera et al. 1984, 1985),

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making it possible to estimate the function of the ASGPR in vivo (Stadlnik et al. 1985; Kudo et al. 1990, 1993). Technetium-99m diethylenetriamine-pentaacetic acid-galactosyl human serum albumin (TcGSA) has been recently developed in Japan and is commercially available for clinical use (Kudo et al. 1991). It has been reported that the Receptor Index (LHL 15), calculated by dividing the radioactivity of the liver region-of-interest (ROI) by that of the liver plus heart ROI 15 min after injection of the radiolabeled ligand, correlated well with classical measures of hepatic function (Kudo et al. 1991). It has been suggested that receptor-binding of a radiopharmaceutical can be a non-invasive indicator of liver function and a measure of functioning hepatocyte mass.

Recently there have been some indications that the hepatocyte ASGPR may be a candidate target antigen inciting the immune reaction of autoimmune hepatitis (AIH) (MacFarlane et al. 1984, 1986; Treichel et al. 1990). Therefore, it is of great interest to evaluate the function of the ASGPR in patients with AIH.

In the present study we report our clinical experience with TcGSA in a variety of chronic liver diseases, including AIH.

Methods

Patients

From February to June 1993, TcGSA studies were performed on normal volunteers and patients with chronic hepatitis C or AIH at our hospital. In chronic hepatitis C group, all patients with chronic hepatitis C had anti-hepatitis C virus (HCV) antibody detected by a second

generation assay and were histologically diagnosed as chronic persistent hepatitis, chronic active hepatitis, or liver cirrhosis. In patients with AIH, the diagnosis was made according to the criteria established by Mackay et al. (1965). All patients diagnosed as having AIH were positive for anti-nuclear antigen with a titer of at least 1:32. At the time of this study, 5 of the 6 patients with AIH were being treated with prednisolone. The TcGSA study was performed on all patients after informed consent was obtained. This project was performed in accordance with the Helsinki Declaration.

Tc-99m DTPA-galactosyl-human serum albumin imaging

All patients received an intravenous injection of Tc-99m GSA (185 MBq) at rest. The patients were placed supine on the imaging table with arms placed comfortably on their forehead. With the patient supine under a rotating gamma camera ZLC-7500 (Shimadzu Corp., Ltd., Kyoto, Japan) equipped with a general all-purpose parallel hole collimator, sequential 20-sec computer-acquired images were obtained for 30 min. The data were recorded in a dedicated computer system using a 64×64 pixel matrix. For liver images, the camera was rotated over 360 degree in an elliptical orbit about the patient's anterior thorax. Data was acquired from 64 views over a 360 degree rotation for 10 sec/view. A 15% window was utilized, centered at the 140 keV photopeak characteristic of technetium 99m. Camera nonuniformity was corrected with the camera's digital flood correction. A circular orbit from a minimal radius of rotation minimized the distance to the patient's thorax.

Quantitative analysis

Liver and entire heart ROIs were identified on the serial images. Time activity curves were generated without 1:2:1 smoothing. The Receptor Index (LHL 15) was calculated from the derivative of the time activity curve. As previously described (Kudo et al. 1991), the Receptor Index was calculated by dividing the radioactivity in the liver ROI by that of the liver plus the heart 15 min after injection. A single investigator (S.I.) measured ROIs in the liver and heart with no information about the patients.

Statistical analysis

Results are expressed as mean $\pm$ SD. The clinical and biochemical data were compared between the autoimmune hepatitis group and the chronic hepatitis C group with the Mann-Whitney U test.

Results

The clinical features of patients in this study are summarized in Table 1. Age, serum albumin concentration, prothrombin time, hepaplastin test, and plasma

TABLE 1.
Clinical features of patients

	AIH group	CVH group
No. of patients	6	8
Age (yr)	47 $\pm$ 15	47 $\pm$ 14
Sex (M/F)	1/5	4/4
Prothrombin time (%)	86.6 $\pm$ 23.3	89.8 $\pm$ 13.4
Hepaplastin test (%)	84.2 $\pm$ 36.4	93.4 $\pm$ 20.6
Albumin (g/L)	36 $\pm$ 4.2	37 $\pm$ 4.0
ICG R 15 (%)	32.5 $\pm$ 4.4	23.8 $\pm$ 11.4

TABLE 2.
Patient profile and the Receptor Index (LHL 15)

Age	Sex	ALT	Dose of Prednisolone	Histological Study	LHL15	Clinical Diagnosis
62	F	21	—	CAH	0.942	CH (type C)
30	F	32	—	CPH	0.937	CH (type C)
43	M	96	—	LC	0.92	LC (type C)
71	M	26	—	CAH with LD	0.903	CH (type C)
37	M	56	—	LC	0.88	LC (type C)
51	F	46	—	CAH	0.846	CH (type C)
37	M	118	—	LC	0.834	LC (type C)
50	F	103	—	CAH with LD	0.811	CH (type C)
47	F	6	5 mg	CAH	0.954	AIH
21	F	67	10 mg	CAH	0.947	AIH
67	M	54	10 mg	LC	0.92	AIH
50	F	34	2.5mg	CAH	0.893	AIH
45	F	86	30 mg	CAH	0.82	AIH
50	F	46	—	LC	0.764	AIH

ALT: alanine aminotransferase; CAH: chronic active hepatitis; CPH: chronic persistent hepatitis; LC: liver cirrhosis; AIH: autoimmune hepatitis; LD: lobular disorganization; CH: chronic hepatitis

retention rate of indocyanine green (ICG) at 15 min were not significantly different between patients with AIH or chronic hepatitis C.

The individual clinical and biochemical data, histological findings, and Receptor Indexes (LHL 15) are shown in Table 2. The clinical diagnosis was as follows: 3 of 8 patients in the chronic hepatitis C group had liver cirrhosis, 4 had chronic active hepatitis and 1 had chronic persistent hepatitis. Six patients had AIH. In the AIH group, 2 patients were histologically diagnosed as having liver cirrhosis and 4 patients were diagnosed as having chronic active hepatitis. Five of the 6 patients in the AIH group were receiving prednisolone. The serum alanine aminotransferase (ALT) level ranged from 6 to 86 (49.7 ± 27.8) in the AIH group, and 21 to 118 (62.3 ± 38.1) in the chronic hepatitis C group. The LHL 15 in 6 normal subjects ranged from 0.946 to 0.965 (0.957 ± 0.008). The LHL 15 in the chronic hepatitis C group ranged from 0.811 to 0.942 (0.884 ± 0.050), and in the AIH group ranged from 0.764 to 0.954 (0.883 ± 0.076). No significant differences were found between the chronic hepatitis C and the AIH groups.

Discussion

In the present study, we assessed the hepatic metabolic activity of the ASGP in patients with AIH or chronic hepatitis C using a radio-labeled ligand. The Receptor Index (LHL 15) introduced by Kudo et al. (1991), which has been reported to be a useful kinetic parameter, was used for this analysis. No significant difference was found between the Receptor Index of

the ASGPR (LHL 15) in patients with AIH and that of patients with chronic hepatitis C.

Nuclear medicine studies, particularly using a receptor-based radiopharmaceutical, can assess tissue function based upon receptor concentration in the target organ (Vera et al. 1984). A new radionuclide liver imaging method using the ASGP receptor was recently developed to assess hepatocyte function (Vera et al. 1984, 1985; Stadalnik et al. 1985; Kudo et al. 1990, 1991, 1993). This TcGSA functional hepatic imaging technique has the additional potential to assess hepatic functional mass. Kudo et al. (1991) have introduced a useful kinetic parameter, Receptor Index (LHL 15). They described that this parameter was acceptable as a substitute for analysis via pharmacokinetic modeling.

MacFarlane et al. (1986) have demonstrated antibodies that recognize the rabbit ASGPR in sera of AIH patients. Treichel et al. (1990) also demonstrated antibodies to human ASGPR in sera of AIH patients. ASGPR has been proposed as a major target antigen of cellular immune reactions in AIH (O'Brien et al. 1986; Löhr et al. 1990). Consequently, ASGPR is one of the most suspectable candidate as target antigen of immune response in AIH patients. If this hypothesis is correct, binding of TcGSA to the ASGPR may be affected in patients with AIH.

Recently, several reports have indicated a high rate of anti-HCV antibody in patients with AIH (MacFarlane et al. 1990; Margrin et al. 1991). Therefore, it is often difficult to differentiate AIH from chronic hepatitis C. Specific therapies now exist for both entities; interferon for chronic hepatitis C (Davis et al. 1989; Di Bisceglie

et al. 1989; Sata et al. 1992) and immunosuppressive therapy for AIH (Soloway et al. 1972; Czaja et al. 1984). Therefore, differentiation of these two diseases is essential for appropriate treatment. If the ASGPR is affected by an immune response in the patients with AIH, the TcGSA study might be a specific screening procedure allowing such a differentiation. Therefore, we felt it would be interesting to assess ASGPR function in patients with AIH. The aim of the present study was to evaluate the TcGSA study as a screening tool for the diagnosis of AIH, and we examined the Receptor Index (LHL 15) in patients with AIH or chronic hepatitis C. These patients were randomly selected, and age and liver function tests were similar in the two groups. No significant difference in LHL 15 was noted in AIH patients vs. patients with chronic hepatitis C. These data do not support the hypothesis that the antibody to the ASGPR might contribute to hepatic disease in patients with AIH. These results, however, may not contradict the previously published hypothesis, because this technique may be of limited sensitivity or differences in the site of recognition between antibodies and ligand may be present. Furthermore, Treichel et al. (1990, 1993) have previously shown that human ASGPR antibody levels closely parallel inflammatory changes in active cases of AIH. In the present study, the majority of patients with AIH were receiving prednisolone, and the inflammatory activity suggested by the serum ALT level was mild. Based on this study, we cannot recommend hepatic functional imaging with TcGSA as a screening method to detect AIH. Further studies are needed to assess the role of an autoim-

mune response against the ASGPR in the pathogenesis of AIH.

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