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Asialoglycoprotein receptor concentration in tumor-bearing livers and its fate early after their sectorial resection

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The aim of the present study was to investigate asialoglycoprotein receptor (ASGP-R) status in tumor-bearing livers and early after their sectorial resection employing ^{99m}Tc-DTPA-galactosyl human serum albumin (^{99m}Tc-GSA) dynamic SPECT. ***Methods:*** Ten normal liver controls and 44 liver tumor patients who underwent sectorial hepatectomy were included in the study. ^{99m}Tc-GSA dynamic SPECT study was performed 7 ± 3 d before (pre-operative) and 34 ± 13 d after surgery (post-operative) in liver tumour patients. Pre- and post-operative parameters including hepatic functional volume and ^{99m}Tc-GSA clearance of unit hepatic functional volume, representing ASGP-R concentration, were measured. The sum of functional volume of the sectors uninvolved in hepatectomy was defined as residual functional volume. Subsequently, post-operative change in functional volume (the ratio of post-operative to residual functional volume), post-operative change in ^{99m}Tc-GSA clearance of unit hepatic functional volume (the ratio of post- to pre-operative ^{99m}Tc-GSA clearance of unit hepatic functional volume) and percent resection of functional volume were calculated. ***Results:*** Pre-operative ^{99m}Tc-GSA clearance of unit hepatic functional volume in tumor-bearing livers was significantly lower than that in non-tumor bearing control liver. The ratio of post- to pre-operative ^{99m}Tc-GSA clearance of unit hepatic functional volume showed marked variation from 0.57 to 2.14, which negatively correlated with the percent resection of functional volume ($r = -0.58$, $p < 0.0001$). The ratio of post- to pre-operative ^{99m}Tc-GSA clearance of unit hepatic functional volume exhibited a negative correlation with the ratio of post-operative to estimated residual functional volume ($r = -0.67$, $p < 0.0001$). ***Conclusion:*** ASGP-R concentration is reduced in the presence of liver tumor. ASGP-R concentration reveals variable changes early after sectorial resection; the change negatively correlates with percent resection of hepatic functional volume. Post-operative change in ASGP-R concentration negatively correlates with change in functional volume.

Key words: asialoglycoprotein receptor, ^{99m}Tc-DTPA-galactosyl human serum albumin, dynamic SPECT, liver neoplasm, liver resection

INTRODUCTION

Asialoglycoprotein receptor (ASGP-R) is a hepatic cell surface receptor specific for galactose-terminated glycoprotein.[1,2] ^{99m}Tc-DTPA-galactosyl human serum albumin (^{99m}Tc-GSA), a newly developed analogous ligand to this receptor, has been used to assess liver function.[3–7] Hepatic receptor imaging involving ^{99m}Tc-GSA enables estimation of ASGP-R amount as serum ^{99m}Tc-GSA clearance, which well correlates with conventional

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indicators of functional capacity.[4–7] ASGP-R concentration (ASGP-R amount in cm^3 liver) is lower in instances of chronic hepatocellular damage than in normal liver.[4] A few reports regarding ^{99m}Tc-GSA receptor imaging have focused on changes in ASGP-R concentration following hepatic resection; however, these data appear controversial. Receptor concentration significantly decreased early after major hepatic resection[7,8]; furthermore, the decrease was more marked in cirrhotic liver.[8] In contrast, other authors demonstrated that post-operative ASGP-R concentration significantly increased in three cases of cirrhotic liver following simple hepatocellular carcinoma (HCC) resection.[9]

In previous investigations, we estimated hepatic functional volume (cm^3, namely, volumetric measurement of functional liver parenchyma) and ^{99m}Tc-GSA clearance of unit hepatic functional volume in terms of ASGP-R concentration (m*l*/min/cm^3 liver) with ^{99m}Tc-GSA dynamic SPECT imaging.[6,7] In the current study, we aim to determine whether the presence of liver tumor influences ASGP-R concentration of the non-tumor parenchyma, how the receptor concentration changes in these patients early following sectorial hepatectomy, and what is the factor affecting the post-operative change of ASGP-R concentration.

MATERIALS AND METHODS

Subjects

Ten normal liver controls and 44 patients presenting with liver tumor and for whom sector(s) resection[10] was planned were included in the study. Control livers were normal on physical examination, computed tomography scan and hepatic functional tests; six subjects were healthy persons and four were patients with non-hepatic disease (small gallbladder tumor in 2, abdominal tumor not associated with the liver in 2); the group comprised 8 men and 2 women; average age ± S.D., 54 ± 15 y. Of the 44 patients, clinical diagnoses were metastatic liver cancer in 16, benign tumor in 4, cholangiocellular carcinoma in 3 and HCC in 21. Post-operative pathological diagnosis of the livers was disease-free parenchyma in 23 patients (men/women [M/F] = 17/6, 56 ± 13 y), chronic hepatitis in 15 (M/F = 14/1, 62 ± 9 y) and cirrhosis in 6 (M/F = 6/0, 58 ± 8 y). Tumor size in diameter was not significantly different among normal parenchyma (5.5 ± 3.5 cm), hepatitis (5.0 ± 4.2 cm) and cirrhosis (4.8 ± 3.2 cm). Pre-operative hepatic function reserve in all patients with chronically diseased liver parenchyma was well preserved, and was classified as Child A (Child-Pugh classification) according to the inpatient records. ^{99m}Tc-GSA dynamic SPECT study was performed 7 ± 3 days before (pre-operative) and 34 ± 13 days after surgery (post-operative) in the 44 patients, but performed only once in controls. All subjects signed an informed consent form based on the guidelines of the institutional human study committee prior to participation in the study.

Dynamic ^{99m}Tc-GSA SPECT image

^{99m}Tc-GSA scintigraphy was conducted as described previously.[7] Briefly, all subjects received 185 MBq of ^{99m}Tc-GSA (3 mg, Nihon Medi-Physics, Nishinomiya, Japan) as a bolus via the antecubital vein. One min after injection, ^{99m}Tc-GSA dynamic SPECT imaging was performed for 15 min employing a triple-head gamma camera (Toshiba GCA9300A, Toshiba, Nasu, Japan) equipped with low-energy high-resolution parallel-hole collimators. The energy discrimination was centered on 140 keV with a 20% window. Fifteen sequential SPECT datasets were acquired. Tomographic images were reconstructed using a ramp filter with a Butterworth filter without attenuation correction. The slice thickness was 2 pixels (1.28 cm).

Imaging analysis

Imaging analysis was also performed as described previously.[7] An ROI was set over the cardiac ventricular cavity (65% cutoff of the maximal count) on the slice demonstrating maximal counts in order to generate an arterial blood time-activity concentration curve. ROIs were also generated over the liver on the last dynamic SPECT images utilizing a 35% cutoff of the maximal count in all slices to estimate the hepatic functional volume (cm^3) per slice; additionally, the sum of the value was calculated as the entire liver functional volume. Nonfunctional liver volume when counts were less than the threshold, e.g. tumor lesions and nonfunctional liver tissue surrounding the lesions, was excluded from functional volume.[7,11] Regional ^{99m}Tc-GSA clearance for each voxel was estimated via the Patlak plot method[12]; the sum of ^{99m}Tc-GSA clearance per voxel was calculated as the total hepatic ^{99m}Tc-GSA clearance (m*l*/min). A ^{99m}Tc-GSA clearance map was also generated. Pre- and post-operative parameters, including functional volume and ^{99m}Tc-GSA clearance of unit functional volume (m*l*/min/cm^3 liver), were measured by ^{99m}Tc-GSA dynamic SPECT.

Pre-operative ^{99m}Tc-GSA functional images were divided into four sectors (left lateral, left medial, right anterior and right posterior) according to the sectorial anatomy of the liver[10] and sectorial mean functional volume and clearance were also estimated. Functional volume of each sector was estimated and the sum of functional volume of the sectors uninvolved in hepatectomy was defined as estimated residual functional volume. Subsequently, percent resection (% resection = [pre-operative functional volume – residual functional volume]/pre-operative functional volume × 100%) was calculated. Post-operative change in ^{99m}Tc-GSA clearance of unit functional volume was calculated by dividing post-operative ^{99m}Tc-GSA clearance of unit functional volume by that of pre-operation. Change in functional volume was also calculated by dividing post-operative functional volume by residual functional volume.

Statistical analysis

Results were reported as mean value ± S.D. The significant differences among the groups were tested by one-way ANOVA followed by Scheffé test. Nonparametric tests (Mann-Whitney test) were applied when appropriate. Linear regression was performed by least-squares analysis. Statistical significance was defined as $p < 0.05$.

RESULTS

The 44 patients were classified into a parenchymal disease-free group (n = 23), a chronic hepatitis group (n = 15) and a cirrhosis group (n = 6) according to the post-operative pathological findings.

^{99m}Tc-GSA clearance of unit functional volume in hepatic tumor patients with disease-free parenchyma, chronic hepatitis and cirrhosis were 0.57 ± 0.22, 0.44 ± 0.09 and 0.49 ± 0.26 m*l*/min/cm^3 liver, respectively, which were significantly lower than that of control (0.89 ± 0.33 m*l*/min/cm^3 liver). The differences in ^{99m}Tc-GSA clearance of unit functional volume among disease-free parenchyma, chronic hepatitis and cirrhosis groups were not statistically significant (Fig. 1A). As depicted in Figure 1B, hepatic functional volume was 1170 ± 151 cm^3, 1171 ± 245 cm^3, 1028 ± 156 cm^3 and 1082 ± 238 cm^3, respectively in controls, hepatic tumor patients with disease-free parenchyma, chronic hepatitis and cirrhosis. Functional volume did not differ significantly among the groups.

The ratio of post- to pre-operative ^{99m}Tc-GSA clearance of unit functional volume showed marked variation from 0.57 to 2.14 after sectorial hepatectomy, which negatively correlated with % resection ($r = -0.58$, $p < 0.0001$, Fig. 2). In instances of greater resection, post-operative ^{99m}Tc-GSA clearance of unit functional volume decreased. On the other hand, post-operative ^{99m}Tc-GSA clearance of unit functional volume exceeded pre-operative levels or remained unchanged when small resection was performed. The ratio of post- to pre-operative ^{99m}Tc-GSA clearance of unit functional volume negatively correlated with the rate of post-operative to residual function volume ($r = -0.67$, $p < 0.0001$, Fig. 3).

Post-operative ^{99m}Tc-GSA clearance of unit functional volume showed variable change, but the ratios of post- to pre-operative ^{99m}Tc-GSA clearance of unit functional volume were similar among the parenchymal disease-free, chronic hepatitis and cirrhotic groups (1.04 ± 0.37, 1.08 ± 0.23, 0.95 ± 0.19, respectively, $p = 0.10$) (Fig. 4). Differences in % resection were not significant ($p = 0.22$) among the groups (40 ± 17%, 31 ± 13% and 30 ± 24%, respectively).

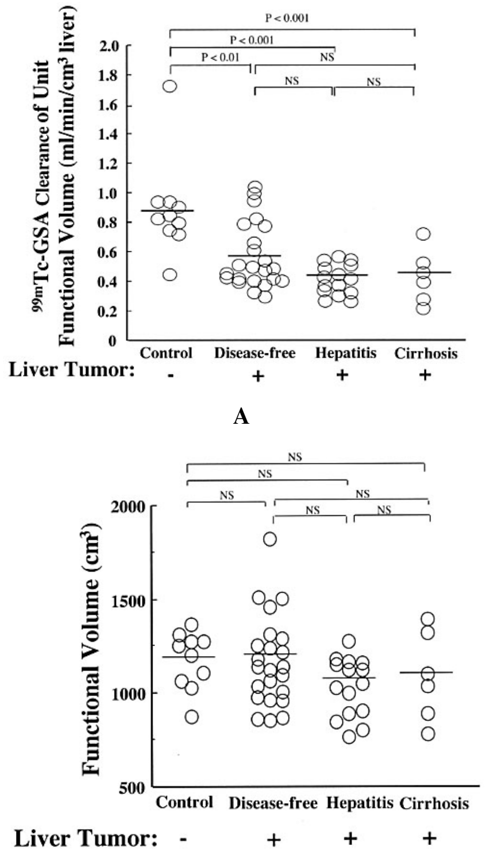

Fig. 1 ^{99m}Tc-GSA clearance of unit hepatic functional volume (A) and hepatic functional volume (B) in controls and in liver tumor patients presenting with different parenchymal disease. +: tumor-bearing; −: tumor-free. Horizontal line represents mean value in each group. Hepatitis = chronic hepatitis, NS = not significant.

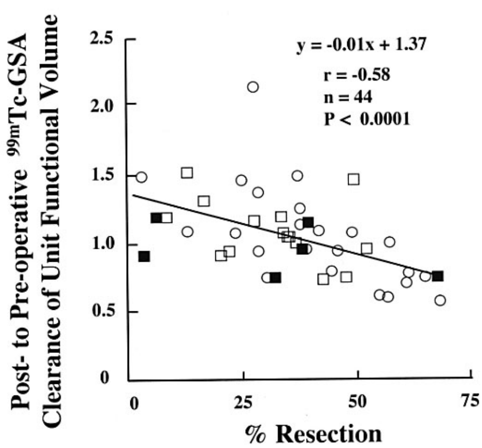

Fig. 2 Correlation between percent resection (% resection) and post-operative change in ^{99m}Tc-GSA clearance of unit hepatic functional volume. (○), Parenchymal disease-free liver; (□), chronic hepatitis liver; (■), cirrhotic liver.

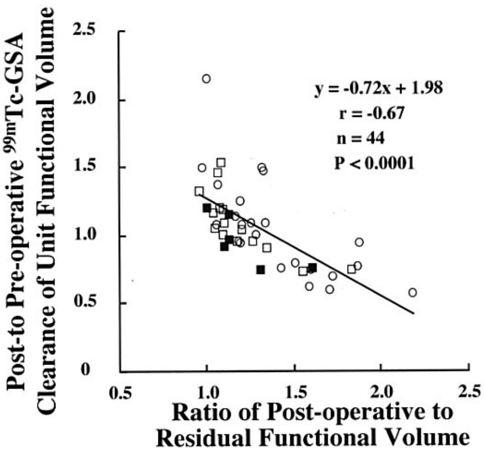

Fig. 3 Correlation between post-operative change in hepatic functional volume and change in ^{99m}Tc-GSA clearance of unit hepatic functional volume. (○), Parenchymal disease-free liver; (□), chronic hepatitis liver; (■), cirrhotic liver.

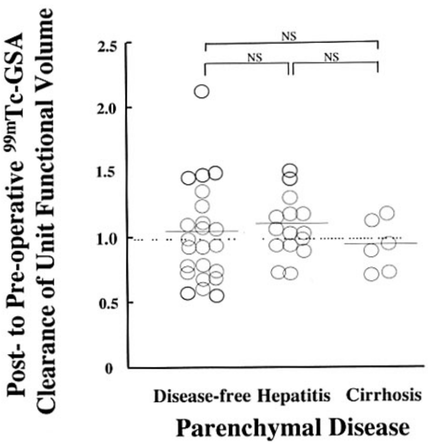

Fig. 4 Comparison of post-operative change in ^{99m}Tc-GSA clearance of unit hepatic functional volume among parenchymal disease-free liver, chronic hepatitis liver and cirrhotic liver. Dotted line indicates the ratio of post- to pre-operative ^{99m}Tc-GSA clearance of unit hepatic functional volume = 1. Horizontal line represents mean value in each group. NS = not significant.

DISCUSSION

Pre-operative ^{99m}Tc-GSA clearance of unit functional volume, the measurement of ASGP-R concentration, in parenchymal disease-free liver was reduced in the presence of liver tumor when compared with controls (Fig. 1A). However hepatic functional volume in tumor-bearing liver did not significantly differ from that in controls (Fig. 1B). The decrease in ASGP-R concentration, therefore, might be explained as preserved functional volume with reduced total ^{99m}Tc-GSA clearance. Tumor expansion might be one of the important factors inducing ASGP-R concentration decrease in tumor bearing liver. In addition, experimental studies suggested that portal flow and portal venous inflow were significantly reduced in the presence of liver tumor.[13] Hence, changes in hemodynamics might also be associated with the reduction of ASGP-R concentration.

Although ASGP-R concentration is lower in instances of chronic hepatocellular damage than in normal liver,[4] the differences in ^{99m}Tc-GSA clearance of unit functional volume among disease-free parenchyma, chronic hepatitis and cirrhosis groups were not statistically significant in our study. This was likely caused by the patient population; namely, the patients with chronic hepatic diseases examined in this study had relatively well preserved hepatic functional reserve.

Post-operative change in ^{99m}Tc-GSA clearance of unit functional volume change correlated negatively with % resection (Fig. 2). Several researchers reported that post-operative ASGP-R concentration measured by the ^{99m}Tc-GSA study decreased in patients who underwent large-range liver resection.[7,8] In contrast, other authors noted that post-operative ASGP-R concentration increased following simple resection of HCC.[9] Both changes were observed in the present investigation.

Our findings indicate that post-operative change in ASGP-R concentration is largely dependent on the post-operative change in functional volume (functional volume regeneration) (Fig. 3). Previous experimental data have demonstrated that hepatocytes are the first cells to proliferate in the early stages following partial hepatectomy to rebuild lost hepatic tissue.[14,15] Newly proliferated hepatocytes contain little ASGP-R, which tends to functional immaturity.[1,14,16] Post-operative decrease in ASGP-R concentration is due to the increased fraction of hepatocytes characterized by little ASGP-R in unit regenerating liver tissue; however, the number of hepatocytes (mature + immature) would not be significantly reduced.[1,14,16] The mechanism of decrease in ^{99m}Tc-GSA clearance of unit functional volume in patients exhibiting quick functional volume regeneration after large resection might be explained by the findings of these previous studies. Obviously, the mechanisms governing the decrease in ASGP-R concentration between regenerating liver and chronically damaged liver appear distinct from each other. Lower ASGP-R concentration in the latter[4] is attributed to a reduction in the number of functional hepatocytes as a consequence of necrosis and fibrosis, whereas functional hepatocytes are intact[17] and ASGP-R levels on single hepatocyte membrane surfaces do not differ from those of normal liver.[18]

On the other hand, we also found that post-operative ^{99m}Tc-GSA clearance of unit functional volume increased in some patients displaying minimal post-operative functional volume regeneration following small resection (Figs. 2, 3). The increase in ^{99m}Tc-GSA clearance of unit functional volume may be based on the elevation in amount (or activity) of ASGP-R on membrane per hepatocyte rather than on a simple increase in hepatocyte number.[19] Increased hepatic blood flow is implied as another possible mechanism.[19] The relationship between post-operative increase in ASGP-R concentration and post-operative hepatic blood flow alteration is unknown and should be pursued in the future.

Some authors demonstrated that ASGP-R concentration greatly decreased in cirrhotic liver early after major hepatic resection[8]; whereas other authors reported that post-operative ASGP-R concentration significantly increased in three cases of cirrhotic liver following simple hepatocellular carcinoma (HCC) resection.[9] In the current study, the change in ASGP-R concentration was not significantly different among groups after a similar extent of resection (Fig. 4), suggesting that liver parenchymal disease is not a major factor affecting post-operative ASGP-R change. It should be noted, however, that since the patients examined in this study had relatively well preserved hepatic functional reserve, our results may not apply to livers with seriously reduced functional reserve. However, because extensive resection of the liver with poor functional reserve (Child B–C) is generally prevented,[10] our results may be of importance in the clinical setting.

In conclusion, ASGP-R concentration is reduced in tumor-bearing liver characterized by both disease-free and chronically diseased parenchyma. Early post-hepatectomized ASGP-R concentration shows variable changes, which negatively correlate with percent resection of hepatic functional volume. In small resection, post-operative ASGP-R concentration exceeded preoperative levels or remained unchanged; in large resection, post-operative ASGP-R concentration decreased. Post-operative change in ASGP-R concentration negatively correlates with change in functional volume.

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