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Stimulation of Liver Regeneration After Hepatectomy in Mice by Injection of Bone Marrow Mesenchymal Stem Cells via the Portal Vein

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ABSTRACT

Aim. To investigate whether mouse bone marrow mesenchymal stem cells (BMC) stimulate liver regeneration after partial hepatectomy.

Methods. Isolated BMCs were purified by density gradient centrifugation. We performed a 70% hepatectomy in male BALB/c mice followed by injection of BMCs into the portal vein (PV-BMC group), or the tail vein (IV-BMC group), or of saline into the portal vein (control group).

Results. The wet weight of the liver remnant increased significantly in the PV-BMC group at 3 and 5 days after hepatectomy compared with the IV-BMC and control groups. The Ki-67 labeling index revealed that the increase to result from stimulation of DNA synthesis. The constitutive interleukin-6 and hepatocyte growth factor mRNAs in the remnant liver tended to increase in the PV-BMC group at 3 days after hepatectomy.

Conclusions. These results demonstrated that BMC injection into the portal vein enhanced liver growth after partial hepatectomy in mice.

AUTOLOGOUS BONE MARROW MESENCHYMAL STEM CELL (BMC) therapy has shown great promise to enhance tissue regeneration in a range of acute and chronic diseases.[1] Prospects for enhanced cardiac regeneration after myocardial infarction have received the greatest attention. The recent 5-year outcome data of BMC transplantation after myocardial infarction demonstrated long-standing improvement in cardiac performance and mortality.[2] BMC therapy has been shown to enhance hepatic regeneration in acute and chronic settings in both preclinical studies and pilot clinical investigations.[3,4] The administration of BMCs following liver resection has not been investigated as yet. Autologous BMC therapy may provide an effective treatment option to facilitate regeneration after liver resection. Administration of autologous BMCs offers a clear advantage over nonautologous cell therapies, as it avoids the requirement for immunosuppression and the risk of sensitization. In this study, we aimed to clarify the role of BMCs in liver regeneration after massive hepatectomy.

MATERIALS AND METHODS

Animals

C57BL/6 (B6) mice were purchased from Shimizu Laboratory Supplies (Shizuoka, Japan). All mice were kept in a pathogen-free room, and 8- to 10-week-old male mice were used in the present study. The university's Committee for Animal Research approved all experiments. BMCs were harvested from the femoral, tibial, and pelvic bones of the mice and suspended in phosphate-buffered saline (PBS). The BMCs were then filtered through a 70-μm nylon wool mesh (Bectone Dickinson Labware, Franklin Lakes, NJ, USA) and centrifuged at 1500 rpm for 7 minutes at 4°C. After centrifugation, the BMCs were suspended and adjusted to 3.0×10^9 cells/mL in PBS containing 2% fetal calf serum.

Seventy percent hepatectomy was performed as previously described by Higgins and Anderson.[5] Briefly, the left lateral, left median, and right median lobes were removed with a single ligature under pentobarbital anesthesia. The mice were divided into three groups: in one group BMCs (3.0×10^7) were injected into the portal vein immediately after hepatectomy (PV-BMC group); in one group BMCs (3.0×10^7) were injected into the tail vein

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immediately after hepatectomy (IV-BMC group); in one group saline was injected into the portal vein immediately after hepatectomy (control group). The livers were excised, weighed, and preserved at 1, 3, or 5 days after hepatectomy (n = 7–10 animals per group for each time point) for subsequent molecular and histological analysis.

Assessment of Liver Regeneration

The weight of regenerated liver was used to calculate the growth of the residual liver lobes, using the formula: weight of regenerated liver/preoperative liver weight × 100 (%). The preoperative liver weight was assumed to be the resected liver weight at hepatectomy × 100/70.

Histology of Liver Tissue

To assess the number of hepatocyte mitoses, liver sections were stained with hematoxylin-eosin, and the percentage of hepatocytes undergoing mitosis was calculated. Liver sections were also incubated with Ki-67 antibody (Novocastra Laboratories Ltd, UK) and the ratio of Ki-67 positive/total hepatocytes was calculated.

mRNA Analysis of Liver Tissue

Total RNA from 50 mg of liver tissue was isolated using TRIzol Reagent (Life Technologies, Rockville, Md, USA) according to the manufacturer's instructions. RNA concentration was determined spectrophotometrically. cDNA was prepared by reverse transcription of 1 mg of total RNA using oligo (dT)18 primer (Biolabs, Frankfurt am Main, Germany) and Superscript II RNaseH-Reverse Transcriptase (Invitrogen, Karlsruhe, Germany). Mouse hepatocyte growth factor (HGF) and interleukin-6 (IL-6) were amplified by polymerase chain reaction (PCR) for 35 to 40 cycles using Taq polymerase (Perkin-Elmer, Rodgau-Jugesheim, Germany). In a comparable assay, RNA integrity and cDNA synthesis were tested using mouse elongation factor (EF)-1α as a housekeeping gene. PCR products were separated by electrophoresis on 2.0% agarose gels. Ethidium-bromide-stained bands were visualized by UV illumination and desitometrically quantified (TotalLab). The data represent expression of HGF and IL-6 gene product in relation to EF-1α.

Statistical Analysis

All data are expressed as the mean ± standard deviation of samples. Statistical analyses were carried out with one-way analysis of variance and significant data were examined by Bonferroni-Dunn multiple comparisons post hoc test. In all cases, a P value < .05 was considered significant.

RESULTS

Liver Regeneration and Ki-67 Labeling Index

Figure 1 shows liver regeneration, expressed as a percentage of the calculated original liver weight. Liver weight increased in the control and IV-BMC groups, reaching about 65% and 70% of the prehepatectomy weight on day 5, respectively. Portal vein injection of BMCs resulted in an approximately twofold increase in weight regeneration over the control and IV-BMC groups on days 3 and 5. The mitotic index was significantly higher in the PV-BMC group than in the control and IV-BMC groups on day 3 after

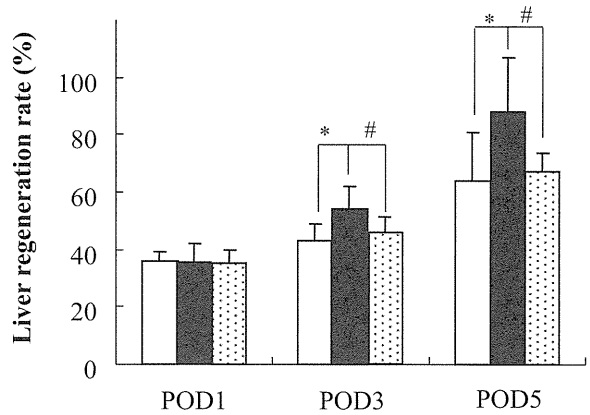

Fig 1. The effect of bone marrow mesenchymal stem cell (BMC) injection on liver regeneration. Liver regeneration in the control group (□), portal vein BMC group (■), and tail vein BMC group (▒) mice are shown as the mean ± standard deviation (n = 7–10). POD, postoperative day. $^{*}P < .05$ versus control; $^{\#}P < .05$ vs tail vein BMC group.

hepatectomy (control, 1.9% ± 1.9%; PV-BMC, 14.1% ± 5.8%; IV-BMC, 6.1% ± 3.0%; P = .0002 between the PV-BMC and control groups, P = .0072 between the PV-BMC and IV-BMC groups). The Ki-67 labeling index was also significantly higher in the PV-BMC group than in the control and IV-BMC groups. These results indicate that portal vein injection of BMCs accelerates liver regeneration in the early period after hepatectomy (control, 9.3% ± 4.7%; PV-BMC, 45.4% ± 17.9%; IV-BMC, 22.8% ± 18.2%; P = .0002 between PV-BMC and control groups, P = .0371 between PV-BMC and IV-BMC groups).

Growth Factors

We analyzed IL-6 and HGF expression by reverse transcriptase PCR in the three groups after hepatectomy. Expression of these growth factors was significantly up-regulated in the PV-BMC group on days 1 and 3 compared with the IV-BMC and control groups (data not shown).

DISCUSSION

The liver has a large capacity for regeneration after resection. However, below a critical level of remnant liver volume, partial hepatectomy is accompanied by a significant increase in postoperative liver failure.[6] Evidence that BMCs contribute to liver regeneration is accumulating.[7,8] Am Esch et al reported that portal vein administration of autologous CD133^{+} BMCs accelerated liver regeneration after clinical portal vein embolization, providing a novel therapy to support hepatic regeneration.[7] Terai and Sakaida reported nine liver cirrhosis cases that underwent autologous bone marrow cell infusion (ABMI) via a peripheral vein and followed their progress for 24 weeks.[8] After ABMI therapy, liver function and Child-Pugh Score were significantly improved at 4 and 24 weeks. There are few

reports describing the administration of BMCs following liver resection. In this study, we evaluated whether injection of BMCs into the portal vein was effective in stimulating liver regeneration after 70% hepatectomy in mice. Our results showed that BMCs stimulated DNA synthesis in hepatocytes and increased the weight of the remnant liver after hepatectomy. We speculate that injection of BMCs into the portal vein accelerates the production of hepatopoietic factors such as IL-6 and HGF in the early period after hepatectomy. The mechanisms by which BMCs may repopulate the regenerating liver are still under discussion. Conversion to liver cells via cell fusion[9,10] or via transdifferentiation without fusion[11] may occur. BMCs may also be a potential source of intrahepatic oval cells, which support liver regeneration.[12] Oval cells are assumed to act as intrahepatic BMCs with the capacity to differentiate into both hepatocytes and bile duct cells.[13–15] In the near future, autologous BMCs may provide a targeted therapy to enhance hepatic regeneration following liver resection, potentially reducing the risks of the procedure.

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Clinical Science

Clinicopathologic characteristics of patients with non-B non-C hepatitis virus hepatocellular carcinoma after hepatectomy

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KEYWORDS:
Liver cancer;
Hepatectomy;
Non-B non-C hepatocellular carcinoma;
Des-gamma-carboxy prothrombin

Abstract

BACKGROUND: A substantial population of hepatocellular carcinoma (HCC) patients is negative for markers of hepatitis B virus and hepatitis C virus (HCV) infection (non-B non-C hepatitis virus [NBC]).

METHODS: Clinicopathologic data and outcomes were compared retrospectively for HCC patients with hepatitis B virus, HCV, and NBC who had undergone hepatectomy.

RESULTS: The TNM stage was significantly higher, and the prevalence of cirrhosis was significantly lower, in the NBC group compared with the HCV group. Among patients with a maximum tumor diameter of 5 cm or less, the survival rates were significantly higher in the NBC group than in the HCV group. Multivariate analysis revealed that preoperative serum des-gamma-carboxy prothrombin (DCP) level was a prognostic factor for survival in NBC–HCC patients. The DCP/tumor size ratio was significantly higher in NBC–HCC patients with normal liver histology than in patients with hepatitis or cirrhosis.

CONCLUSIONS: NBC–HCC patients had more advanced tumors compared with HCV–HCC patients, but significantly higher survival rates. Measurement of DCP potentially is significant for early diagnosis of NBC HCC, which may increase the chance of curative therapy without recurrence.

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide.[1] Although most HCC cases are still found in Asia and Africa, recent studies have shown that the incidence of HCC, and mortality resulting from HCC, are increasing in North America and Europe.[2,3] Evidence shows that 50% of all cases of HCC worldwide are associated with hepatitis B virus (HBV) infection, with a further 25% associated with hepatitis C virus (HCV).[4–6] Some recent reports have shown that the primary risk factor for developing HCC is cirrhosis.[4–8] The annual incidence rates of HCC in patients with cirrhosis resulting from HBV and HCV are reported as 1% to 8% and 1% to 15%, respectively.[6–8] The oncogenic mechanisms and the clinicopathologic characteristics of HCC strongly are influenced by HBV or HCV infection.[9–11] For example, patients with HBV-related HCC have a shorter history of infection and better liver function reserve than those with HCV-related HCC. HCV antibody (HCVAb)-positive HCC accounts for more than 80% of all cases of HCC in Japan, and these patients are characteristically older and have more severe

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cirrhosis than those with hepatitis B surface antigen (HBsAg)-positive HCC. The prognosis of HCVAb-positive HCC is worse than that of HBsAg-positive HCC[9] because multicentric carcinogenesis is more common in patients with HCV infection than in patients with HBV infection,[10,12,13] and the outcome after resection differs between patients with HBV and HCV infection.[14–16]

Although most HCC is associated with viral infection, there is a substantial population of HCC patients (5%–15%) who are negative for markers of HBV and HCV infection (non-B non-C hepatitis virus [NBC]) in Japan and Taiwan.[10,11,17–22] Although several studies have compared the clinicopathologic features of patients with NBC HCC and viral-related HCC, controversy remains about the liver function of NBC-HCC patients, the biological behavior of their tumors, and the outcome after surgical treatment. In the present study, we retrospectively analyzed NBC-HCC patients who had undergone potentially curative resection to determine the risk factors for recurrence after hepatectomy and to help clarify the appropriate treatment for this type of HCC.

Materials and Methods

Subjects

Between February 1992 and January 2009, a total of 534 patients with HCC underwent curative resection (defined as macroscopic removal of all tumor) at our institution. Of these, 19 patients died before hospital discharge and the remaining 515 were followed up as outpatients. We excluded 7 patients with both HBsAg and HCVAb, 1 patient with autoimmune hepatitis, 1 patient with primary biliary cirrhosis, and 10 patients with alcoholic cirrhosis. The remaining 496 patients were divided into the following 3 groups: the HBV-HCC group (n = 85), which were positive for HBsAg and negative for HCVAb; the HCV-HCC group (n = 351), which were negative for HBsAg and positive for HCVAb; and the NBC-HCC group (n = 60), which were negative for both HBsAg and HCVAb.

Clinicopathologic variables and surgery

Before surgery, each patient underwent conventional liver function tests, measurement of the indocyanine green retention rate at 15 minutes (ICGR15), and technetium-99m-diethylenetriamine-pentaacetic acid-galactosyl-human serum albumin liver scintigraphy.[23] Hepatitis virus screening was performed by measurement of HBsAg and HCVAb. α-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) levels were measured in all patients. Surgical procedures were classified according to the Brisbane terminology proposed by Strasberg et al.[24] Anatomic resection was defined as resection of the tumor together with the related portal vein branches and corresponding hepatic territory, and was classified as hemihepatectomy (resection of half of the liver), extended hemihepatectomy (hemihepatectomy plus removal of additional contiguous segments), sectionectomy (resection of 2 Couinaud subsegments[25]), or segmentectomy (resection of 1 Couinaud subsegment). All other procedures were classified as limited resection, which frequently was performed for peripheral or central tumors. Peripheral tumors and those with extrahepatic growth were treated by partial hepatectomy because this procedure achieved a sufficient surgical margin. Central tumors located near the hepatic hilum or major vessels were treated by enucleation only because it was too difficult and/or dangerous to remove enough liver tissue to obtain an adequate margin. One senior pathologist reviewed all specimens for histologic confirmation of the diagnosis. The width of the surgical margin was measured as the distance from the tumor edge to the resection line.

Follow-up evaluation

Perioperative and postoperative complications and deaths were recorded to determine morbidity and mortality after hepatectomy.

All patients who survived were followed up at least every 3 months after discharge. Follow-up evaluation included physical examination, liver function tests, chest radiographs to check for pulmonary metastases, and ultrasonography, computed tomography, or magnetic resonance imaging to check for intrahepatic recurrence. Chest computed tomography was performed if the chest radiograph showed any abnormalities. Bone metastases were diagnosed by bone scintigraphy.

When recurrence of HCC was detected by changes in tumor markers or on imaging, recurrence limited to the remnant liver was treated by transarterial chemoembolization, lipiodolization, re-resection, or percutaneous local ablative therapy such as radiofrequency ablation. When extrahepatic metastases were detected, active treatment was undertaken in patients with good hepatic functional reserve (Child–Pugh class A or B) and good performance status (0 or 1), whereas other patients were given only radiation therapy to relieve symptoms of bone metastases. Surgical resection was undertaken in patients with a solitary extrahepatic metastasis and no intrahepatic recurrence.

Prognostic factors

We performed univariate and multivariate analyses of 30 clinicopathologic factors to identify independent variables related to postoperative disease-free survival and overall survival of NBC–HCC patients. The patient factors we investigated were sex, age, body mass index, alcohol abuse, the presence or absence of diabetes mellitus, and liver function (including albumin, total bilirubin, aspartate aminotransferase, alanine aminotransferase, prothrombin time, cholinesterase, platelet count, alkaline phospha-

tase, ICGR15, maximal removal rate of GSA, and Child–Pugh class). The tumor factors investigated were AFP level, DCP level, histologic features (including tumor diameter, differentiation, microscopic capsule formation, surgical margin, and vascular invasion), the number of tumors, and stage according to the TNM classification.[26] The surgical factors investigated were surgical time, blood loss, perioperative blood transfusion, surgical procedure, and complications. All the variables that were significant according to univariate analysis then were examined with a Cox proportional hazards model to identify variables that had an independent influence on disease-free survival or overall survival.

Statistical analysis

Continuous variables are presented as mean ± standard deviation. The significance of differences among the 3 groups was assessed by the chi-square test, the Mann–Whitney *U* test, or the Kruskal–Wallis test as appropriate. Correlations between various tumor factors and the DCP/tumor size ratio were determined by the Pearson correlation coefficient analysis. The Kaplan–Meier method was used to calculate the disease-free survival rate and overall survival rate as of June 2009, and the significance of differences in survival rates was estimated with the generalized log-rank test. The Cox regression model (stepwise method) was used for multivariate analysis. In all analyses, a P value less than .05 was considered statistically significant.

Results

Preoperative characteristics

Table 1 summarizes the preoperative characteristics of the 3 groups of HCC patients. Patients in the NBC–HCC group had a significantly higher body mass index and a higher prevalence of diabetes mellitus than patients in the HBV–HCC and HCV–HCC groups. The mean age of the patients in the HBV–HCC group was significantly lower than that of the NBC–HCC and HCV–HCC groups. There were no significant differences in the prevalence of preoperative alcohol abuse among the 3 groups. The NBC–HCC and HBV–HCC groups had significantly better preoperative liver function (serum albumin, alanine aminotransferase, platelet count, and ICGR15) than the HCV–HCC group. The NBC–HCC and HBV–HCC groups had significantly higher AFP and DCP levels than the HCV–HCC group.

Perioperative parameters and pathologic findings

Table 2 shows that surgical time, blood loss, and blood transfusion did not differ significantly among the 3 groups.

Table 1 Preoperative clinical characteristics of the 3 groups of HCC patients[1]

	NBC–HCC group (n = 60)	HBV–HCC group (n = 85)	HCV–HCC group (n = 351)
Sex, male/female	52/8	68/17	272/79
Age, y	66.6 ± 13.3	59.3 ± 11.4*	66.5 ± 7.1
BMI, kg/m^2	24.2 ± 3.6†	22.8 ± 3.2	22.4 ± 2.8
Alcohol abuse, +/−	30/30	40/45	139/212
Diabetes, +/−	25/35‡	21/64	81/270
Child-Pugh class A/B	54/6	74/11	317/34
ICGR15, %	15.3 ± 10.9	14.6 ± 10.2	20.0 ± 9.9§
Platelet count, $10^4/\mu L$	18.5 ± 9.5	18.1 ± 7.2	13.5 ± 6.6‖
Total bilirubin level, mg/dL	.78 ± .31	.80 ± .63	.88 ± .33¶
Albumin level, g/dL	3.84 ± .53	3.81 ± .45	3.68 ± .43#
Prothrombin time, %	92 ± 14	89 ± 14	88 ± 13**
ALT level, U/L	33 ± 21	44 ± 36	59 ± 39††
AFP, ng/mL	2,246 ± 8,584	15,600 ± 55,009	454 ± 3,250‡‡
DCP, mAU/mL	3,755 ± 9,726	4,655 ± 12,379	1,527 ± 6,464§§

Data represent the mean ± standard deviation or the number of patients.
ALT = alanine aminotransferase; BMI = body mass index.
*P = .0006 and <.0001 versus NBC–HCC and HCV–HCC patients.
†P = .0108 and <.0001 versus HBV–HCC and HCV–HCC patients.
‡P = .0307 and .0024 versus HBV–HCC and HCV–HCC patients.
§P = .0388 and <.0001 versus NBC–HCC and HBV–HCC patients.
‖P < .0001 and <.0001 versus NBC–HCC and HBV–HCC patients.
¶P = .0480 versus NBC–HCC patients.
#P = .0126 and .0178 versus NBC–HCC and HBV–HCC patients
**P = .0325 versus NBC–HCC patients.
††P < .0001 and .0016 versus NBC–HCC and HBV–HCC patients.
‡‡P = .0045 and <.0001 versus NBC–HCC and HBV–HCC patients.
§§P = .0245 and .0014 versus NBC–HCC and HBV–HCC patients.

Table 2 Intraoperative and postoperative characteristics of the 3 groups of HCC patients

	NBC–HCC group (n = 60)	HBV–HCC group (n = 85)	HCV–HCC group (n = 351)
Surgical time, min	319 ± 119	305 ± 114	288 ± 111
Surgical blood loss, mL	1,498 ± 1,450	1,538 ± 2,410	1,465 ± 1,736
Blood transfusion, +	28 (47%)	37 (44%)	145 (41%)
Surgical procedure			
Limited resection	40 (67%)	47 (55%)	290 (83%)*
Anatomic resection	20 (33%)	38 (45%)	61 (17%)
Patients with complications, n	10 (17%)	7 (8%)	86 (25%)†
Tumor size, cm	5.57 ± 4.58	5.43 ± 4.03	3.55 ± 2.48‡
Tumor differentiation			
Well or moderate	56 (93%)	74 (87%)	304 (87%)
Poor or necrosis	4 (7%)	11 (13%)	47 (13%)
Microscopic surgical margin, +	3 (5%)	12 (14%)	38 (11%)
Microvascular invasion, +	26 (43%)	48 (56%)	154 (44%)
Tumors, n			
Single	50 (83%)	59 (69%)	262 (75%)
Multiple	10 (17%)	26 (31%)	89 (25%)
Underlying liver histology			
Normal	24 (40%)	11 (13%)	14 (4%)
Hepatitis	21 (35%)	51 (60%)	181 (52%)
Cirrhosis	15 (25%)	23 (27%)§	156 (44%)‖
Tumor stage (TNM)			
I or II	32 (53%)	43 (51%)	249 (71%)
III or IV	28 (47%)	42 (49%)	102 (29%)¶

Data represent the mean ± standard deviation or the number of patients.
NS = not significant.
*P = .0041 and <.0001 versus NBC–HCC and HBV–HCC patients.
†P = .001 versus HBV–HCC patients.
‡P < .0001 and <.0001 versus NBC–HCC and HBV–HCC patients.
§P = .0005 versus NBC–HCC patients.
‖P < .0001 and .0004 versus NBC–HCC and HBV–HCC patients.
¶P = .0067 and .0003 versus NBC–HCC and HBV–HCC patients.

The NBC–HCC and HBV–HCC groups had a lower percentage of limited resection cases than the HCV–HCC group. On pathologic examination, tumor size was significantly larger and TNM stage was significantly more advanced in the NBC–HCC and HBV–HCC groups than in the HCV–HCC group. Normal liver histology was significantly more common in the NBC–HCC group than in the other 2 groups.

Outcome

The disease-free survival and overall survival rates of all 496 patients were 35% and 73% at 3 years, 22% and 57% at 5 years, and 11% and 26% at 10 years, respectively (Fig. 1). There were significant differences in disease-free survival rates between the NBC–HCC or HBV–HCC groups and the HCV–HCC group (45% in NBC–, 41% in HBV–, and 33% in HCV–HCC patients at 3 years; 35%, 32%, and 17% at 5 years; and 30%, 29%, and 11% at 7 years, respectively; P = .0395), although there were no significant differences in overall survival rates among the groups (75% in NBC, 66% in HBV, and 74% in HCV–HCC patients at 3 years; 62%, 53%, and 57% at 5 years; and 58%, 37%, and 44% at 7 years, respectively; P = .2123). Among patients with a maximum tumor diameter of 5 cm or less, disease-free survival rates were significantly higher in the NBC–HCC and HBV–HCC groups than in the HCV–HCC group (P = .0003 and P = .0073, respectively) (Fig. 2A), and

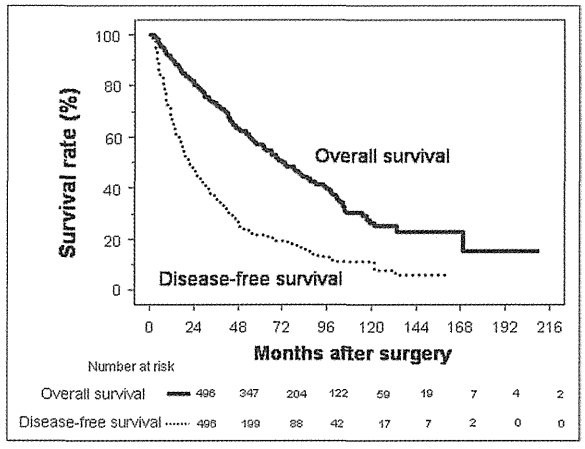

Figure 1 Disease-free and overall survival rates in all 496 patients after hepatectomy for HCC.

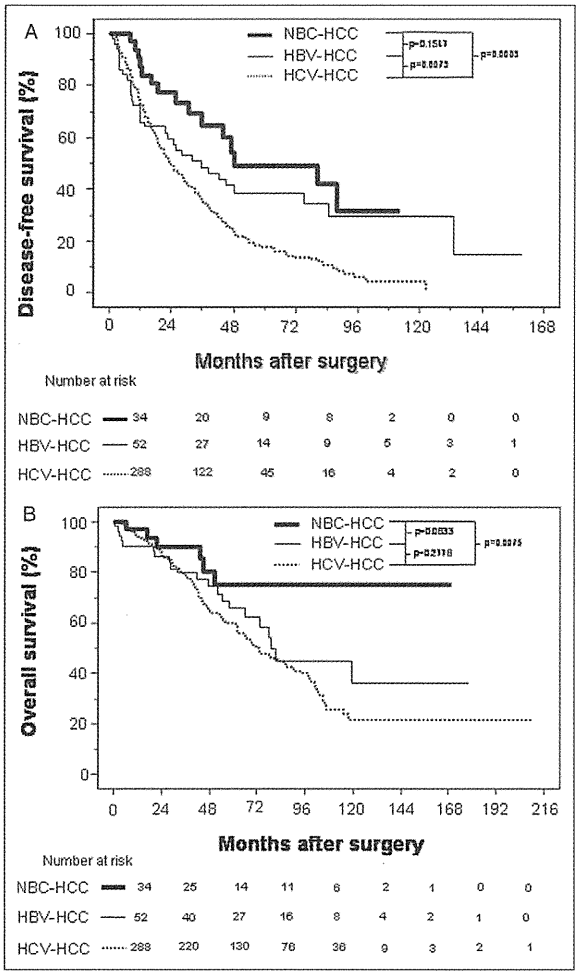

Figure 2 Comparisons of disease-free survival and overall survival rates after hepatectomy among patients in the HBV–HCC, HCV–HCC, and NBC–HCC groups with a maximum tumor diameter of 5 cm or less. (A) Disease-free survival. The survival rate of HCV–HCC patients (dotted line) was significantly lower than that of NBC–HCC (unbroken thick line, $P = .0003$) or HBV–HCC patients (unbroken thin line, $P = .0073$). (B) Overall survival. The survival rate of NBC–HCC patients (unbroken thick line) was significantly better than that of HCV–HCC patients (dotted line, $P = .0075$). The numbers of patients at risk are shown below each graph.

there was also a significant difference in overall survival rate between the NBC–HCC group and the HCV–HCC group ($P = .0075$) (Fig. 2B).

Factors affecting disease-free survival and overall survival rates

Univariate analysis showed that the factors associated with lower disease-free survival in the NBC–HCC group were sex, serum albumin level, DCP level, number of tumors, microscopic vascular invasion, and TNM stage, whereas the factors associated with lower overall survival were serum albumin level, total bilirubin level, prothrombin time, DCP level, and complications. Table 3 shows the results obtained by multivariate analysis (Cox proportional hazards model) of factors with an influence on disease-free survival or overall survival rates. A serum albumin level less than 3.9 g/dL, a DCP level of 300 mAU/mL or greater, and multiple tumors were identified as independent prognostic indicators of disease-free survival, whereas a serum albumin level less than 3.9 g/dL and a DCP level of 300 mAU/mL or greater had an independent influence on overall survival.

Correlations between tumor factors and DCP/tumor size ratio or underlying liver histology in NBC–HCC patients

There were significant correlations between tumor size and both AFP and DCP levels in our 496 patients (AFP: $r = .347$, $P < .0001$; DCP: $r = .562$, $P < .0001$). We divided the AFP and DCP levels by tumor diameter to calculate tumor marker/tumor size ratios. The DCP/tumor size ratio was correlated positively with tumor stage ($r = .34$; $P = .0091$), number of tumors ($r = .392$; $P = .0026$), tumor histology ($r = .42$; $P = .0018$), and microvascular invasion (MVI) ($r = .546$; $P < .0001$), whereas the AFP/tumor size ratio was not (data not shown).

Correlations between perioperative factors and underlying liver histology were analyzed in the NBC–HCC group (normal histology, n = 24; hepatitis, n = 21; or cirrhosis, n = 15). There were significant correlations between MVI and normal histology (MVI with normal histology, n = 17; hepatitis, n = 5; and cirrhosis, n = 4; $P = .0021$), and between anatomic resection and normal histology (anatomic resection with normal histology, n = 16; hepatitis, n = 4; and cirrhosis, n = 0; $P < .0001$). There were no significant correlations between histology and blood loss, blood transfusion, tumor stage, number of tumors, or tumor differentiation.

Correlations between tumor marker/tumor size ratios and underlying liver histology

The AFP/tumor size ratio in the NBC–HCC group was 316 ± 918 ng/mL/cm, 50 ± 101 ng/mL/cm, and 842 ± 581 ng/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The AFP/tumor size ratio in the HBV–HCC group was 3,175 ± 3,241 ng/mL/cm, 1,774 ± 4,346 ng/mL/cm, and 394 ± 691 ng/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The AFP/tumor size ratio in the HCV–HCC group was 43 ± 89 ng/mL/cm, 107 ± 537 ng/mL/cm, and 73 ± 160 ng/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. There were no significant correlations between the AFP/tumor size ratio and underlying histology in the NBC–HCC, HBV–HCC, or HCV–HCC groups.

Table 3 Prognostic factors for disease-free survival and overall survival identified by multivariate analysis in NBC–HCC patients

Variable	Coefficient	Standard error	Relative risk	*P* value
Disease-free survival				
Albumin level, <3.9 g/dL	1.110	.414	3.034	.0074
DCP, ≥300 mAU/mL	.923	.396	2.959	.0063
Multiple tumors	.749	.446	3.802	.0028
Overall survival				
Albumin level, <3.9 g/dL	1.560	.647	4.761	.0159
DCP, ≥300 mAU/mL	.813	.541	2.778	.0389

The DCP/tumor size ratio in the NBC–HCC group was 757 ± 1,142 mAU/mL/cm, 146 ± 270 mAU/mL/cm, and 132 ± 153 mAU/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The DCP/tumor size ratio in the HBV–HCC group was 719 ± 867 mAU/mL/cm, 692 ± 1,734 mAU/mL/cm, and 489 ± 654 mAU/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. The DCP/tumor size ratio in the HCV–HCC group was 363 ± 464 mAU/mL/cm, 301 ± 934 mAU/mL/cm, and 135 ± 756 mAU/mL/cm with normal histology, hepatitis, and cirrhosis, respectively. There were no significant correlations between the DCP/tumor size ratio and underlying histology in the HBV–HCC and HCV–HCC groups. In contrast, the DCP/tumor size ratio was significantly higher in NBC–HCC patients with normal histology than with hepatitis or cirrhosis ($P = .0211$ and $P = .0426$, respectively).

Comments

Although most HCC still occurs in patients with persistent HCV infection, the incidence of HCV HCC has been decreasing over the past years because of the promotion of anti-HCV therapy[27] and a decrease in the number of patients with chronic HCV infection.[28] In recent reports, the percentage of HCC patients with NBC HCC has reached as high as 20%, and the incidence is increasing.[10,11,17–22,29] These findings indicate that NBC HCC may become more important in the near future.

We found that NBC–HCC patients were significantly older than HBV–HCC patients, but not HCV–HCC patients, which is consistent with a previous report by Dohmen et al.[30] It seems that although most HBV–HCC cases result from vertical transmission of HBV in infancy, causing HCC at a young age, NBC HCC develops over a long time period later in life.[31] Abe et al reported that the most important etiologic factor for the development of NBC HCC is alcohol consumption, followed by nonalcoholic fatty liver disease.[29] However, we did not find a higher prevalence of alcohol abuse among NBC–HCC patients in the present study. Comparisons among the 3 groups found that preoperative liver function was best in the NBC–HCC group. The smaller tumors and lower levels of AFP and DCP in the HCV–HCC group may reflect periodic screening for HCC in these patients. Normal liver histology was significantly more common in the NBC–HCC group than in the HCV–HCC or HBV–HCC groups. Assessment of liver histology revealed a lower grade of inflammation and earlier stage of fibrosis in the NBC–HCC group. The increased frequency of anatomic resection in the NBC–HCC group may have been related to the better liver function compared with the HCV–HCC group.

Tumor size, multiple tumors, portal invasion, and curative resection with an adequate surgical margin have been reported as prognostic indicators in NBC–HCC patients undergoing resection.[32,33] In the present study, we found that a lower serum albumin level and a higher DCP level were independent predictors of disease-free survival and overall survival in the NBC–HCC group. There has been no previous report that the preoperative serum DCP level is a prognostic indicator for NBC HCC. According to Yamamoto et al,[34] although both AFP and DCP increase with tumor growth, DCP is a more accurate tumor marker. In most NBC–HCC patients, the tumor is discovered at an advanced stage[17–22] because these patients have milder hepatic dysfunction and fewer symptoms compared with HBV–HCC and HCV–HCC patients owing to the absence or mildness of underlying chronic liver disease. Because they have fewer symptoms, NBC–HCC patients visit the hospital less frequently than HBV–HCC or HCV–HCC patients, and thus undergo imaging studies less frequently. NBC HCC usually is not discovered until the tumor is large enough to cause abdominal pain and distension.

We found significant differences in disease-free survival rates, but not overall survival rates, among the 3 groups. However, both disease-free and overall survival rates of NBC–HCC patients after hepatic resection were significantly higher if the maximum tumor diameter was 5 cm or less. Several studies have indicated that large tumors, especially those greater than 5 cm in size, have a significantly higher risk of recurrence.[35–38] The influence of size is attributed to the increased invasiveness of larger tumors, as shown by a higher incidence of intrahepatic metastases and portal venous invasion.[39,40] Therefore, in HCC patients with larger tumors, survival depends on progression of the tumor itself (including its size and/or number of lesions) irrespective of the type of underlying hepatitis virus infection. Although NBC–HCC patients with normal liver histology often had advanced tumors with microvascular invasion, these patients showed higher survival rates owing to curative anatomic resection and good residual liver function. We

also found that measurement of DCP level in the perioperative and postoperative periods may be a useful prognostic indicator. If measurement of DCP level in NBC–HCC patients could lead to earlier diagnosis of the primary tumor or of recurrence after surgical resection, the use of DCP levels for screening may improve prognosis.

In conclusion, we found that the DCP/tumor size ratio of NBC–HCC patients was correlated positively with several tumor factors, including tumor stage, the number of tumors, tumor histology, and microvascular invasion. NBC–HCC patients had significantly higher survival rates after hepatic resection than HBV–HCC or HCV–HCC patients because they generally had milder hepatic dysfunction. The DCP/tumor size ratio was significantly higher in NBC–HCC patients with normal histology than those with hepatitis or cirrhosis. These findings should be considered when treating NBC–HCC patients. Further prospective studies are required to fully evaluate the significance of DCP level in NBC patients with potentially curable HCC.

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INVITED REVIEW ARTICLE

Usefulness of Tc-99m-GSA scintigraphy for liver surgery

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Abstract Postoperative mortality remains high after hepatectomy compared with other types of surgery in patients who have cirrhosis or chronic hepatitis. Although there are several useful perioperative indicators of liver dysfunction, no standard markers are available to predict postoperative liver failure in patients with hepatocellular carcinoma (HCC) undergoing hepatectomy. The best preoperative method for evaluating the hepatic functional reserve of patients with HCC remains unclear, but technetium-99m diethylenetriamine pentaacetic acid galactosyl human serum albumin (^{99m}Tc-GSA) scintigraphy is a candidate. ^{99m}Tc-GSA is a liver scintigraphy agent that binds to the asialoglycoprotein receptor, and can be used to assess the functional hepatocyte mass and thus determine the hepatic functional reserve in various physiological and pathological states. The maximum removal rate of ^{99m} Tc-GSA (GSA-Rmax) calculated by using a radiopharmacokinetic model is correlated with the severity of liver disease. There is also a significant difference of GSA-Rmax between patients with chronic hepatitis and persons with normal liver function. Regeneration of the remnant liver and recurrence of hepatitis C virus infection in the donor organ after living donor liver transplantation have also been investigated by ^{99m}Tc-GSA scintigraphy. This review discusses the usefulness of ^{99m}Tc-GSA scintigraphy for liver surgery.

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Keywords GSA-Rmax · Hepatocellular carcinoma · Hepatectomy · Living donor liver transplantation · Regeneration · Hepatitis C virus

Introduction

Ashwell and Morell [1] demonstrated the existence of a hepatic receptor for asialoglycoprotein (ASGP) during investigation of ceruloplasmin metabolism. They found that ceruloplasmin molecules lacking a sialic acid residue disappeared rapidly from the circulation and were taken up by hepatocytes [2]. The ASGP receptor is only expressed by mammalian hepatocytes, and is almost always expressed on the sinusoidal and lateral surfaces of hepatocytes in the normal liver [3]. Sawamura et al. [4] reported that a decrease of ASGP receptors led to accumulation of ASGP in the serum of galactosamine-treated rats. Expression of this receptor is also decreased in patients with chronic liver disease [5]. Technetium-99m diethylenetriamine pentaacetic acid galactosyl human serum albumin (^{99m}Tc-GSA) is a liver scintigraphy agent that binds to the ASGP receptor on hepatocytes [6]. The maximum removal rate of Tc-GSA (GSA-Rmax) calculated with a radiopharmacokinetic model is reported to decrease as liver disease becomes more severe, and there is also a significant difference of GSA-Rmax between patients with chronic hepatitis and persons with normal liver function [7]. Because this agent binds to hepatocytes for a long period, the distribution of the functioning hepatocyte mass can be assessed by performing single-photon emission computed tomography (SPECT) with Tc-GSA [8]. Hepatic abnormalities detected by ^{99m}Tc-GSA scintigraphy show a good correlation with histologic abnormalities, especially steatosis and fibrosis or necrosis in patients with fatty liver or chronic hepatitis

[9, 10]. This review focuses on the use of ^{99m}Tc-GSA to measure preoperative hepatic function in HCC patients undergoing hepatectomy, as well as the hepatic functional reserve in donors and recipients after living donor liver transplantation (LDLT), as reported previously by Kaibori et al. [11–13].

Evaluation of preoperative hepatic function in patients with hepatocellular carcinoma undergoing hepatectomy

Hepatocellular carcinoma (HCC) is the fifth most common cancer worldwide [14]. Although the majority still occurs in Asia and Africa, the incidence and mortality rate of HCC have recently been increasing in North America and Europe [15, 16]. In Japan, most HCCs are associated with chronic hepatitis and liver cirrhosis induced by hepatitis B or hepatitis C virus infection. Due to advances in perioperative management, anesthesia, and operative techniques, the performance of hepatectomy for HCC has become more common. However, the postoperative mortality rate remains high in patients who have cirrhosis or chronic hepatitis compared with that for other types of surgery. In fact, the mortality rate after major hepatectomy is between 5 and 21% for patients with cirrhosis [17–21]. The postoperative course does not always proceed as predicted because of various intraoperative stresses, including blood loss and ischemia, so preoperative evaluation of hepatic function in HCC patients undergoing hepatectomy is essential. Several perioperative variables, including the galactose elimination capacity [22], preoperative portal pressure [23], ^{99m}Tc-GSA liver scintigraphy [7], indocyanine green (ICG) clearance test [24, 25], amino acid clearance test [26], and aminopyrine breath test [27], are useful for identifying hepatic impairment in patients with HCC undergoing hepatectomy. Some studies have indicated that ICG clearance (expressed as the percentage of ICG retained at 15 min) is the best preoperative test for evaluation of the hepatic functional reserve in HCC patients [24, 25]. However, discrepancies between ICG clearance and liver histology are occasionally seen, which are thought to mainly depend on the effective hepatic blood flow resulting from intrahepatic and extrahepatic shunts. ICG has been considered an ideal substance for kinetic analysis of hepatic function since it is nontoxic at clinical doses and is reported to not undergo extrahepatic removal, intrahepatic conjugation, or enterohepatic circulation. ICG is a near-infrared fluorescent dye that was approved by the US Food and Drug Administration for cardiovascular and liver function diagnostic testing. There have also been recent reports about the usefulness of ICG for intraoperative fluorescence imaging to detect sentinel nodes in patients with breast cancer or gastric cancer [28, 29]. We and others have found that HCC shows very strong fluorescence in patients who have been given ICG several days before surgery for routine preoperative assessment of liver function (Fig. 1) [30, 31]. Therefore, we came to doubt that the ICG clearance test is the best procedure for evaluating preoperative hepatic function, because not only did ICG dye show intrahepatic conjugation, but it also accumulated in the HCC nodules.

The ICGR15 test and ^{99m}Tc-GSA scintigraphy were performed in 384 patients with HCC prior to liver resection at our institution. Table 1 shows the correlations between GSA-Rmax or ICGR15 and other laboratory test results in HCC patients with a preoperative ICGR15 $< 20\%$. There were significant correlations between GSA-Rmax or ICGR15 and other laboratory values. However, only GSA-Rmax, and not ICGR15, was significantly correlated with some of the laboratory tests in HCC patients with a preoperative ICGR15 $\geq 20\%$ (Table 2). Thus, both ^{99m}Tc-GSA scintigraphy and ICG clearance may be useful

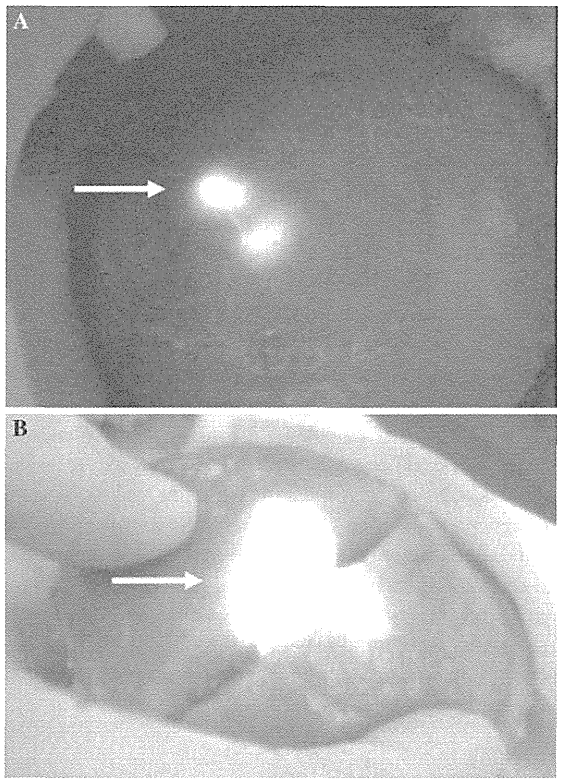

Fig. 1 Indocyanine green fluorescence imaging in a 62-year-old woman who underwent hepatectomy for HCC. **a** Intraoperative ICG fluorescence imaging shows a strong signal in the primary HCC nodule (*thin arrow*). **b** Postoperative ICG fluorescence imaging shows a strong signal in the primary tumor nodule in the liver slice (*thin arrow*)

Table 1 Correlations between GSA-Rmax or ICGR15 and other laboratory tests in hepatocellular carcinoma patients with ICGR15 <20%

Test	GSA-Rmax			ICGR15		
	n	*r*	*P* value	*n*	*r*	*P* value
ICGR15	236	−0.397	**<0.0001**			
AST	236	−0.362	**<0.0001**	236	0.199	**0.0021**
Total bilirubin	236	−0.291	**<0.0001**	236	0.356	**<0.0001**
Platelet count	236	0.48	**<0.0001**	236	−0.328	**<0.0001**
Albumin	236	0.09	0.17	236	−0.035	0.5961
Cholinesterase	236	0.413	**<0.0001**	236	−0.222	**0.0006**
Prothrombin time	234	0.183	**0.0049**	234	−0.159	**0.0151**
Type IV collagen 7S	119	−0.231	**0.0112**	119	0.209	**0.0225**
Hyaluronate acid	124	−0.299	**0.0007**	124	0.060	0.5091

Significant differences are shown in bold

ICGR15 indocyanine green retention rate at 15 min, *AST* aspartate aminotransferase, *GSA-Rmax* regional maximum removal rate of technetium-99m galactosyl human serum albumin

Table 2 Correlations between GSA-Rmax or ICGR15 and other laboratory tests in hepatocellular carcinoma patients with ICGR15 ≥ 20%

Test	GSA-Rmax			ICGR15		
	n	*r*	*P* value	*n*	*r*	*P* value
ICGR15	148	−0.107	0.1969			
AST	148	−0.137	0.098	148	−0.044	0.6001
Total bilirubin	148	−0.12	0.1474	148	0.283	**0.0005**
Platelet count	148	0.303	**0.0002**	148	−0.505	0.5507
Albumin	148	0.413	**<0.0001**	148	−0.125	0.1308
Cholinesterase	148	0.496	**<0.0001**	148	−0.117	0.1557
Prothrombin time	148	0.375	**<0.0001**	148	−0.067	0.4204
Type IV collagen 7S	93	−0.306	**0.0027**	93	0.196	0.0593
Hyaluronate acid	94	−0.316	**0.0018**	94	0.133	0.2009

Significant differences are shown in bold

ICGR15 indocyanine green retention rate at 15 min, *AST* aspartate aminotransferase, *GSA-Rmax* regional maximum removal rate of technetium-99m galactosyl human serum albumin

procedures for preoperative evaluation of the hepatic functional reserve in patients with HCC.

Hyaluronate/GSA-Rmax ratio as a predictor of postoperative liver failure

The serum levels of type IV collagen and hyaluronic acid (HA) were measured in 191 patients with HCC prior to liver resection, and ^{99m}Tc-GSA scintigraphy was also performed. In brief, 3 mg of Tc-GSA (185 MBq; Nihon Medi-Physics, Nishinomiya, Japan) was injected into an antecubital vein as a bolus dose. Images were obtained as 10-s frames for 15 min after injection using a gamma camera with a large field of view (GSA-7100A/DI; Toshiba, Tokyo) and a high-resolution, parallel-hole collimator centered over the liver and precordium. Then GSA-Rmax was calculated by using a radiopharmacokinetic model [7].

Liver failure was defined by the postoperative occurrence of any of the following: encephalopathy associated with hyperbilirubinemia (total bilirubin >5 mg/dl) for more than 5 days, intractable pleural effusion or ascites (requiring diuretics, thoracocentesis, or abdominal paracentesis on 2 or more occasions, or continuous drainage), or variceal bleeding [31, 32]. Logistic regression analysis was performed and odds ratios (ORs) were calculated to estimate the relative risk of postoperative liver failure. In these analyses, $P < 0.05$ was considered to indicate statistical significance.

Postoperative liver failure occurred in 16 patients (encephalopathy associated with hyperbilirubinemia in 3

Table 3 Risk factors for hepatic failure after resection of hepatocellular carcinoma calculated by multivariate analysis

GSA-Rmax regional maximum removal rate of technetium-99m-galactosyl human serum albumin, *HA* hyaluronic acid, *AFP* α-fetoprotein, *CI* confidence interval

Variable	Odds ratio	95% CI	*P* value
Albumin < 3.7 g/dl	4.12	0.85–20.00	0.0796
Total bilirubin ≥ 0.7 mg/dl	4.13	0.44–38.60	0.2134
GSA-Rmax < 0.475 mg/min	0.17	0.01–2.92	0.2229
Type IV collagen 7S ≥ 6.0 ng/ml	0.13	0.01–1.27	0.0792
HA ≥ 150 ng/ml	1.54	0.13–18.81	0.7338
Type IV collagen 7S/GSA-Rmax ≥ 15 mg min/dl	7.65	0.31–36.14	0.2116
HA/GSA-Rmax ≥ 500 mg min/dl	23.60	1.91–62.09	0.0138
AFP ≥ 17 ng/ml	4.14	0.78–21.99	0.0951

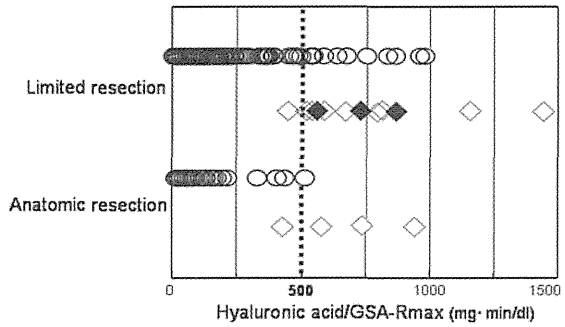

Fig. 2 Relations among the HA/GSA-Rmax ratio, surgical procedure, and occurrence of liver failure. *Circles* patients without postoperative liver failure. *Open diamonds* patients with postoperative liver failure. *Closed diamonds* patients who died of postoperative liver failure

patients, refractory massive ascites or pleural effusion in 12 patients, and variceal bleeding in 1 patient), and three of them died of liver failure in hospital. The ORs of possible risk factors for postoperative liver failure calculated by univariate analysis were as follows: age (OR = 3.56), Child-Pugh class B (OR = 3.33), ICGR15 (OR = 3.25), serum albumin (OR = 6.21), total bilirubin (OR = 10.74), cholinesterase (OR = 3.40), platelet count (OR = 4.93), AST (OR = 4.80), GSA-Rmax (OR = 8.13), type IV collagen 7S (OR = 3.97), HA (OR = 11.85), type IV collagen 7S/GSA-Rmax ratio (OR = 18.08), HA/GSA-Rmax ratio (OR = 21.49), AFP (OR = 4.54), microscopic invasion of the portal vein and/or hepatic vein (OR = 3.65), and cirrhosis (OR = 3.12). According to multivariate analysis, an HA/GSA-Rmax ratio ≥500 mg min/dl (OR = 23.60; 95% CI = 1.91–62.09; $P = 0.0138$) was the only independent predictor of postoperative liver failure (Table 3). The HA/GSA-Rmax ratio was significantly higher in patients with postoperative liver failure than in patients without it after either anatomic resection or limited resection (both $P < 0.0001$, Fig. 2). Following limited resection, all of the patients who died of postoperative liver failure had an HA/GSA-Rmax ratio ≥500 mg min/dl (Table 4).

Other reported risk factors for postoperative liver failure include type IV collagen 7S [33, 34], HA [35, 36], and GSA-Rmax [7], but these parameters were not found to be significant in our study. To reduce postoperative liver failure, various measures of the hepatic functional reserve should be assessed before surgery, including tests of both parenchymal and nonparenchymal liver function. Limited liver resection has been recommended for the treatment of HCC in patients with cirrhosis [37, 38]. Three patients with a HA/GSA-Rmax ratio ≥500 mg min/dl who underwent limited resection died of postoperative liver failure in our study. Our findings indicate that the HA/GSA-Rmax ratio is a useful predictor of the risk of postoperative liver failure and a ratio ≥500 mg min/dl is a relative contraindication to hepatectomy.

Liver regeneration in donors evaluated by ^{99m}Tc-GSA scintigraphy after living donor liver transplantation

When living donor liver transplantation (LDLT) is performed, steatosis is one of the risk factors for graft dysfunction and severe macrovesicular steatosis is an absolute contraindication to transplantation [39]. Hepatic steatosis is also reported to affect the postoperative recovery of the donor [40]. However, the extent of macrovesicular steatosis has been reported to decrease immediately after partial hepatectomy, and although early liver regeneration is impaired after partial hepatectomy in patients with mild macrovesicular steatosis, long-term regeneration is reported to be normal [41]. Thus, whether mild hepatic steatosis influences regeneration of the donor's liver after partial hepatectomy is still controversial. Accordingly, we employed ^{99m}Tc-GSA scintigraphy to assess the impact of steatosis on regeneration and function of the remnant donor liver after hepatectomy.

A total of 14 patients underwent LDLT at our institution and 12 living donors with complete ^{99m}Tc-GSA liver scintigraphy data and histological data from intraoperative liver biopsy specimens were investigated. The liver-to-spleen CT attenuation ratio (*L/S* ratio) was measured on

Table 4 Changes of serum HCV RNA, HAI, METAVIR score, and liver function parameters after transplantation

Time (mo)	Pre	1	3	6	12
Patient 1					
HCV RNA (IU/ml)	1.5×10^6	1.5×10^6	3.2×10^6	3.8×10^6	6.8×10^6
HAI score	ND	ND	3	5	10
METAVIR score	ND	ND	A1/F0	A2/F1	A2/F2
Hyaluronic acid (ng/ml)	912	ND	229	265	306
ALT (U/L)	39	93	62	26	37
Platelet count (10^4/ml)	6.1	16.4	9.3	14.2	8.0
Prothrombin time (%)	50	72	77	84	75
Total bilirubin (mg/dl)	6.1	1.3	1.6	1.6	1.5
Patient 2					
HCV RNA (IU/ml)	0.3×10^6	0.6×10^6	3.7×10^6	3.8×10^6	3.5×10^6
HAI score	ND	ND	2	4	4
METAVIR score	ND	ND	A1/F0	A1/F0	A1/F1
Hyaluronic acid (ng/ml)	378	ND	136	76	69
ALT (U/L)	36	90	115	128	104
Platelet count (10^4/ml)	5.7	6.6	12.2	8.6	9.1
Prothrombin time (%)	49	89	100	91	94
Total bilirubin (mg/dl)	3.6	8.0	1.6	0.9	0.6
Patient 3					
HCV RNA (IU/ml)	0.4×10^6	1.3×10^6	7.0×10^6	4.9×10^6	0.5×10^{6a}
HAI score	ND	ND	4	10	22[a]
METAVIR score	ND	ND	A1/F1	A2/F2	A3/F4[a]
Hyaluronic acid (ng/ml)	218	ND	523	411	ND
ALT (U/L)	47	85	53	100	91[a]
Platelet count (10^4/ml)	4.0	7.2	5.4	4.9	4.6[a]
Prothrombin time (%)	70	88	83	79	52[a]
Total bilirubin (mg/dl)	2.4	2.5	2.2	2.7	24.0[a]
Patient 4					
HCV RNA (IU/ml)	0.7×10^5	2.5×10^6	6.1×10^6	0.3×10^4	0.4×10^6
HAI score	ND	ND	3	3	4
METAVIR score	ND	ND	A1/F0	A1/F0	A1/F1
Hyaluronic acid (ng/ml)	300	ND	82	103	94
ALT (U/L)	40	17	43	46	18
Platelet count (10^4/ml)	2.5	8.5	9.6	7.2	8.7
Prothrombin time (%)	37	73	87	71	87
Total bilirubin (mg/dl)	3.6	1.6	1.7	1.3	1.0

[a] This was determined at 8 months after transplantation. Treatment with a combination of interferon plus ribavirin was initiated at 7 months after transplantation

HAI histologic activity index, *A* activity score, *F* fibrosis score, *ALT* alanine aminotransferase, *ND* not determined

noncontrast CT scans as an index of hepatic steatosis, as described previously [42]. Liver biopsy specimens obtained during surgery were assessed for macrovesicular steatosis, which was classified as absent (0%), mild (<30%), moderate (30–60%), or severe (>60%). The median *L/S* ratio for each of these histological categories was 1.20, 1.12, 1.01, and 0.90, respectively, with the optimum *L/S* ratio for prediction of mild or moderate hepatic steatosis being 1.20 and 1.10, respectively. The donors were classified into 2 groups with or without mild hepatic steatosis according to the *L/S* ratio, i.e., 6 donors who had an *L/S* ratio $\geq$1.20 (mean $\pm$ standard deviation, 1.35 ± 0.03) were assigned to the control group, whereas the other 6 donors who had an *L/S* ratio <1.20 (mean $\pm$ standard deviation, 1.10 ± 0.11) were assigned to the fatty liver group. Informed consent was obtained from all of the donors. To perform ^{99m}Tc-GSA scintigraphy, 3 mg of Tc-GSA (185 MBq, Nihon Medi-Physics,

Nishinomiya, Japan) was injected into an antecubital vein as a bolus dose. Images were obtained as 10-s frames for 15 min after injection using a gamma camera with a large field of view (GCA-7100A/DI, Toshiba, Tokyo, Japan) and a high-resolution, parallel-hole collimator centered over the liver and precordium. Two quantitative indices were calculated from the time-activity curves thus obtained. The blood clearance index was calculated as the ratio of uptake by the heart at 15 min to that at 3 min (HH15), and the hepatic accumulation index was calculated as the ratio of uptake by the liver alone to that of uptake by the liver plus heart at 15 min (LHL15) [43]. Then these two indices were used to calculate the hepatic uptake ratio corrected by blood clearance (LHL/HH) as a parameter of the hepatic functional reserve. Taking the values of the LHL/HH ratio, GSA-Rmax, and computed tomographic liver volume (CT-LV) before partial hepatectomy as 100%, the results obtained at 1, 3, 6, and 12 months after surgery were expressed as a percentage of the preoperative values. This revealed that the CT-LV of the fatty liver group and the control group was respectively 72 ± 3 and 78 ± 8% of the baseline value at 1 month after partial hepatectomy, whereas it was 79 ± 8 and 81 ± 12% at 3 months, 82 ± 5 and 83 ± 6% at 6 months, and 85 ± 5 and 90 ± 9% at 12 months (Fig. 3a). There were no significant differences between the two groups at any time.

The fatty liver group had a significantly lower LHL/HH ratio and GSA-Rmax than the control group at both 6 and 12 months after hepatectomy (Fig. 3b, c). The LHL/HH ratio and GSA-Rmax were decreased at 1 month in both groups, but these values returned more rapidly to baseline in the control group and GSA-Rmax even became higher than before hepatectomy. In the fatty liver and control groups, the LHL/HH ratio and GSA-Rmax were respectively 79 ± 7 versus 95 ± 8% and 83 ± 2 versus 125 ± 24% of baseline at 1 year after surgery.

Recently, LDLT has become an alternative to cadaveric liver transplantation as a means of overcoming the perpetual shortage of donor organs. The unique ability of the liver to regenerate completely after resection makes this approach possible. A recent massive increase in the number of partial liver transplant procedures has renewed interest in liver regeneration. The process of regeneration ceases after the liver has achieved 75–95% of its original weight. Pomfret et al. [44] reported that regeneration achieved an average of 84 ± 9.0% of the original liver volume by 1 year after surgery. Humar et al. [45] reported that recipients showed a greater increase of liver volume than their living donors, with the donor livers reaching 79% of their original volume by 3 months, postoperatively. We previously reported that the regenerated liver volume estimated by CT volumetry was significantly correlated with that estimated by ^{99m}Tc-GSA after partial

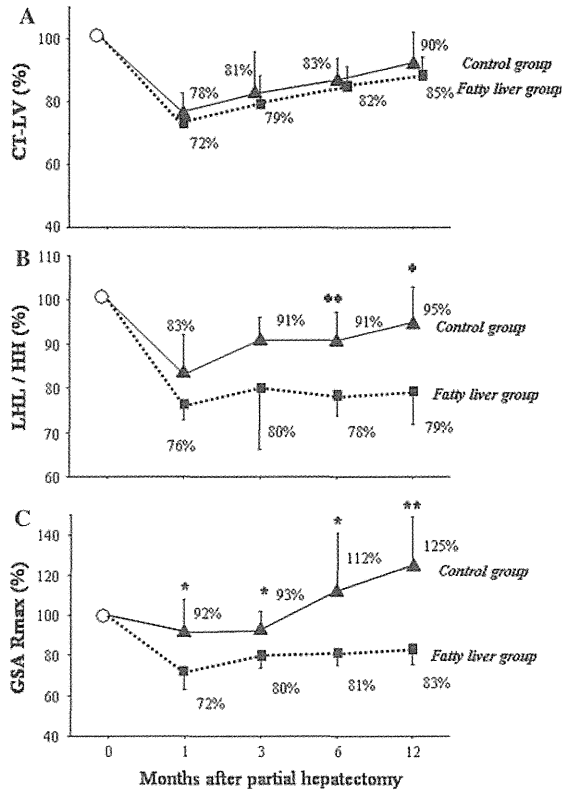

Fig. 3 Changes of CT-LV, LHL/HH, and GSA-Rmax after partial hepatectomy. **a** CT-LV, **b** LHL/HH, and **c** GSA-Rmax. Each value obtained before hepatectomy was set at 100%, and the values obtained at 1, 3, 6, and 12 months after surgery were expressed as percentages of the preoperative value

hepatectomy for benign and malignant tumors [46]. Our study also showed that normal livers (control group) underwent rapid regeneration and reached 90 ± 9% of the preoperative volume by 1 year after hepatectomy (Fig. 4a). A certain amount of redundancy built into the liver may explain why regeneration tends to cease before the pretransplant volume is reached. In our study, the LHL/HH ratio and GSA-Rmax were decreased at 1 month after surgery in both groups, but both parameters returned to prehepatectomy levels by 1 year in the control group, whereas the fatty liver group had a significantly lower LHL/HH ratio and GSA-Rmax at 6 and 12 months after hepatectomy (Fig. 4b, c). These results indicate that regeneration of functioning hepatocytes is impaired when the donor has mild hepatic steatosis. Our findings also suggest that careful management of major or minor morbidities is required during the regeneration period after partial hepatectomy in donors with mild hepatic steatosis.

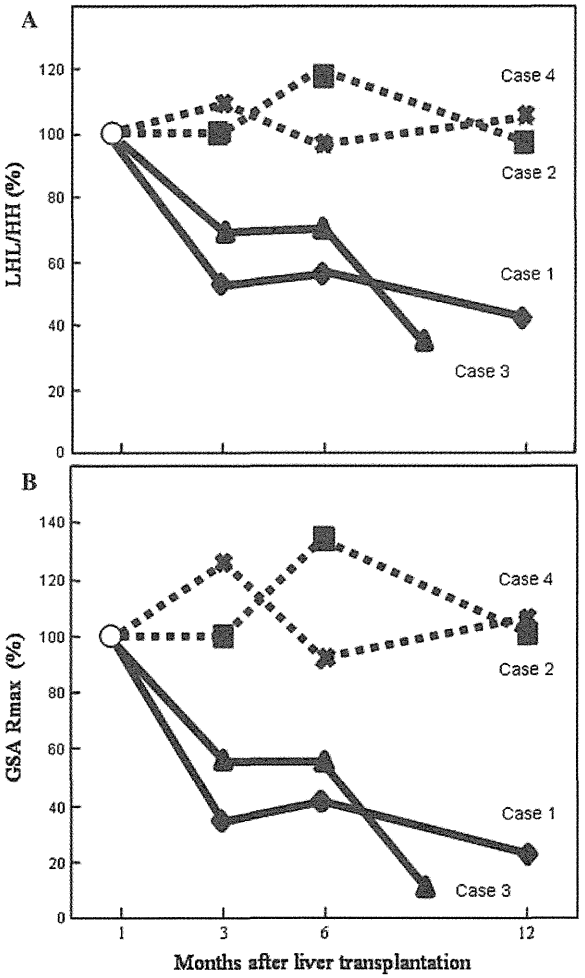

Fig. 4 Changes of LHL/HH and GSA-Rmax after transplantation. **a** LHL/HH, **b** GSA-Rmax. The values of LHL/HH and GSA-Rmax obtained at 1 month after transplantation were set as 100%, and the values obtained at 3, 6, and 12 months were expressed as a percentage of the 1-month values

Recurrent hepatitis C after living donor liver transplantation detected by ^{99m}Tc-GSA liver scintigraphy

Hepatitis C virus (HCV)-related liver disease is the leading indication for liver transplantation [47]. Recurrent hepatitis that causes cirrhosis or graft loss respectively occurs in approximately 20 and 10% of patients within 5 years after transplantation [48, 49], and the risk of these complications increases over time [50]. Because the number of liver transplant recipients with recurrent and severe hepatitis C has continued to increase, various HCV treatment strategies have been explored. At present, mainstream therapy involves providing treatment for patients with histologically progressive liver disease after transplantation. However, the overall response rate achieved with a combination of interferon plus ribavirin is approximately 20%, indicating that this approach is unlikely to be successful in the majority of patients [51]. Unfortunately, the optimum timing for initiation of treatment and the dosages of interferon and ribavirin required to achieve viral eradication after liver transplantation have not yet been established. Although liver biopsy is essential to determine the need for interferon and ribavirin therapy, this is an invasive procedure, particularly in the early post-transplant period. We investigated whether recurrent hepatitis C could be detected by ^{99m}Tc-GSA scintigraphy after LDLT.

A total of 14 patients underwent LDLT at our institution. Among them, six patients had decompensated cirrhosis because of hepatitis caused by HCV infection. Two of these six patients did not undergo ^{99m}Tc-GSA scintigraphy after transplantation, and were excluded. The remaining 4 patients were reviewed retrospectively after informed consent was obtained. Before surgery, the four patients were shown to have HCV genotype 1b. All patients tolerated surgery well without significant intraoperative or early postoperative complications and recovered uneventfully. They received a standard primary immunosuppression protocol consisting of tacrolimus and corticosteroids [52]. All four patients underwent laboratory tests and assessment of graft function by ^{99m}Tc-GSA scintigraphy at 1, 3, 6, and 12 months after transplantation. Percutaneous liver biopsy was also performed at 3, 6, and 12 months after surgery. The diagnosis of hepatitis was based on the histologic activity index (HAI) [53] and the METAVIR [54, 55] score, which demonstrates the grade of necroinflammation and stage of fibrosis. Taking the LHL/HH ratio and GSA-Rmax at 1 month after transplantation as 100%, the values at 3, 6, and 12 months were expressed as a percentage of the 1-month values. Table 3 shows the changes of serum HCV RNA, HAI, METAVIR score, and liver function parameters after transplantation in each patient. HCV RNA was detected in the serum at 3 months after transplantation in all four patients. In patient 1, HCV RNA was twofold higher at 12 months compared with its level at 6 months. In patients 3 and 4, serum HCV RNA was decreased at 6 and 8 months compared with the level at 3 months. The levels of hyaluronic acid and alanine aminotransferase (ALT), as well as the prothrombin time and platelet count, showed little change after transplantation in all of the patients. Although total bilirubin was decreased at 6 months in patients 2 and 4, it did not decrease in patients 1 and 3. At 6 months after transplantation, the HAI score determined by liver biopsy was 5 in patient 1 and 10 in patient 3. The METAVIR activity score (A) was 2 and the fibrosis score (F) was 1 in patient 1, whereas the

scores were A2/F2 in patient 3. In patient 1, the HAI and METAVIR score respectively showed an increase to 10 and A2/F2 at 12 months after transplantation, and recurrence of HCV infection was also indicated by other histologic findings. Accordingly, the patient was treated with a combination of interferon plus ribavirin. In patient 3, treatment with a combination of interferon plus ribavirin was initiated at 7 months after transplantation, but the HAI and METAVIR score respectively increased to 22 and A3/F4 at 8 months. The liver histology was compatible with fibrosing cholestatic hepatitis and the patient died of progressive hepatic failure at 10 months after transplantation. In patients 2 and 4, recurrence of HCV infection was not detected by histologic examination. The LHL/HH ratio and GSA-Rmax showed little change after transplantation in these patients (Fig. 4). In patient 1, however, the LHL/HH ratio and GSA-Rmax both showed a decrease at 3 months after transplantation and subsequently remained at low levels. Both the LHL/HH ratio and GSA-Rmax were decreased at 3 months after transplantation in case 3, and declined further by 8 months.

This study showed that changes of ^{99m}Tc-GSA scintigraphy were better correlated with the stage of hepatic fibrosis than with the grade of necroinflammation (as indicated by the METAVIR score). We previously reported that the results of ^{99m}Tc-GSA scintigraphy were well correlated with the HAI score, especially that for fibrosis, and suggested that scintigraphy may be useful for noninvasive preoperative evaluation of hepatic fibrosis [7]. Because hepatic fibrosis causes the loss of normal hepatocytes and reduces the number of receptors available to bind ^{99m}Tc-GSA, scintigraphy can indicate the pathological stage of chronic hepatic parenchymal damage. In our study, recurrent HCV infection was confirmed by histologic examination at 6 and 12 months after transplantation in two patients. The decrease of the LHL/HH ratio and GSA-Rmax at 3 months after transplantation in these two patients suggested an early effect of recurrent hepatitis C on the graft before changes of other parameters occurred. It may be reasonable to commence antiviral therapy on the basis of such findings. In patients with recurrent hepatitis C after LDLT, accurate evaluation of hepatic functional reserve is very important for selection of treatment and estimation of the prognosis, but a standard method has not been established. Although needle biopsy is currently essential to determine the indications for interferon and ribavirin therapy, this test is quite invasive in the early posttransplantation period. Thus, ^{99m}Tc-GSA liver scintigraphy may have a potential role as a noninvasive method of evaluating graft functional reserve, but its value will need to be confirmed by further investigations.

Conclusion

To reduce postoperative liver failure, preoperative planning should employ various tests to assess the hepatic functional reserve, including tests of both parenchymal and nonparenchymal liver function. The HA/GSA-Rmax ratio can predict liver failure after hepatectomy, and a ratio ≥500 mg min/dl is a relative contraindication to liver resection. After LDLT, ^{99m}Tc-GSA liver scintigraphy may be useful for evaluating the regeneration of functioning hepatocytes. Because we found that donors with mild hepatic steatosis showed impaired liver regeneration at 1 year after partial hepatectomy, management of such donors requires more care. In liver transplant recipients with HCV, a decrease of GSA-Rmax at 3 months after transplantation suggests recurrent HCV infection of the graft, and ^{99m}Tc-GSA liver scintigraphy is a useful noninvasive method for evaluating the graft functional reserve.

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