was up- regulated in chronic active hepatitis.[22,23] These adhesion molecules, especially ELAM-1, were reported to mediate the adhesion of HepG2 cells and the serum ICAM-1 levels also increased after a hepatectomy.[23] The up-regulation of adhesion molecules and hepatitic activity could enhance early intrahepatic metastasis from primary HCCs. Adachi et al.[17] reported that hepatic resection especially in the active hepatitis group might give rise to various grades of impaired immunity. If HCC cells were spread in the remnant liver during and/or after resection, it would be easy for them to survive and proliferate in the liver of patients with such an impaired immunity. Taking together, early intrahepatic recurrence after curative resection of HCCs seems to be associated with not only primary tumor factors but also underlying hepatitic activity.

On the other hand, the significant risk factor for late recurrence in the present study was only the underlying hepatic status such as the presence of HPF, but neither primary tumor factors nor operative factors. The livers with HPF had higher hepatitic activity (higher Grading, and/or Staging) and higher scores of PCNA L.I. than HPF-negative ones. Hyperplastic foci are analogous to parts of adenomatous hyperplasias (AHs) or early HCCs.[11] Wakasa et al.[24] reported that the proliferative activity of HPF, as expressed according to the PCNA L.I., is almost the same as that of AHs and early HCCs. They also reported that HPF-positive livers had a higher hepatitic activity and HPF lesions reflected the risk of multicentric hepato-carcinogenesis. Tarao et al.[9] and Koike et al.[10] reported that HCC development was significantly linked to underlying liver diseases with a high degree of DNA synthesis, suggesting that inflammatory liver tissues has a potential to develop HCCs. Therefore, it is possible that HPF itself may be one of the precancerous lesions in multicentric carcinogenesis.

In conclusions, not only the tumor factors but also the underlying hepatic status including Grading, Staging, and the presence of HPF were significant risk factors for the intrahepatic recurrence after curative resection for HCC. To achieve better outcome in HCC patients after curative resection, anti-hepatitis treatments as well as anti-tumor treatments during pre- and post-operative phase should be established.

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Original Article

Hepatitis C virus kinetics during the first phase of pegylated interferon-α-2b with ribavirin therapy in patients with living donor liver transplantation

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Aim: To identify the problems of pegylated interferon (PEG IFN) with ribavirin therapy against hepatitis C virus (HCV) reinfection in living donor liver transplantation (LDLT) patients. HCV kinetics during the PEG IFN with ribavirin therapy were analyzed in LDLT patients, as well as in chronic hepatitis C (CHC) patients.

Methods: The study included 80 consecutive HCV infected patients undergoing PEG IFN with ribavirin therapy (64 CHC and 16 LDLT patients) who attended the Nagasaki University Hospital for an initial visit between January 2005 and December 2007.

Results: The sustained viral response (VR) rate of the CHC group (80%) was superior to the LDLT group (22%). The viral disappearance rate of the CHC group was also superior to the LDLT group, regardless of the HCV serotype. The HCV core antigen (cAg) titer under treatment in the LDLT group was more than that of the CHC group from day 0 to week 12. The HCV cAg decrease rate of the LDLT group on the first day of treatment was less than that of the CHC group.

Conclusion: The HCV infection of a transplanted liver is more refractory to treatment than a non-transplanted liver. The low reduction HCV cAg rate on day 1 is one of the problems of the combination therapy.

Key words: chronic hepatitis C, first phase, hepatitis C virus, interferon, living donor liver transplantation

INTRODUCTION

HEPATITIS C VIRUS (HCV) infection is widespread throughout the world. Chronic HCV infection leads to cirrhosis and hepatocellular carcinoma. Liver transplantation for HCV-related liver disease has been an option worldwide.[1] Recently, it has been shown that the prognosis for liver transplanted (LT) patients with HCV-related disease deteriorates over time,[2] thus resulting in a poorer outcome than in the non-HCV course.[3] The transplanted liver for HCV-related disease undergoes a rapidly progressive fibrosis and acute graft failure.[3,4] Consequently, anti-HCV treatment after LT is important for the prognosis. Interferon (IFN) has been recognized as the only treatment method for HCV infection. For the transplanted liver, it is known that IFN treatment improves liver fibrosis or halts the progression.[5] Recently, the combination of pegylated IFN (PEG IFN) with ribavirin was used and produced an excellent result for non-transplanted patients with HCV.[6] However, that was not the case for the HCV re-infected transplanted liver.[7] It is important that the cause of refractory HCV infection in the transplanted liver be more fully clarified. Immunosuppressant therapy, especially with glucocorticoid, has been speculated to be the cause of the refractory nature of the transplanted liver to IFN.[8,9] The cause of this is considered to be that glucocorticoid downregulated the IFN signal transduction in the hepatocytes.[8] The authors recently found that calcineurin inhibitors also inhibited IFN induced STAT-1 phosphorylation and antiviral activity in the HCV

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replicon system.[10] Therefore, the problem of IFN signaling in the hepatocyte induced an IFN refractory condition[11] and decreased the first phase of HCV decline, which was IFN induced HCV decay during the first day of IFN treatment.[12]

In the present study, we attempted to better understand PEG IFN and ribavirin therapy by comparing patients with chronic hepatitis from HCV infection (CHC) with living donor LT (LDLT) patients. When the non-transplanted CHC patients were used as a reference against the HCV reinfected LDLT patients, we expected that the differences in the clinical data in the two groups would help to clarify the problem of IFN refractory HCV infection, and shed light on the analysis of HCV kinetics under IFN and ribavirin treatment, and to elucidate the damaged segment of the IFN induced antiviral mechanism in the LDLT condition.

PATIENTS AND METHODS

Patients

THE PRESENT RESEARCH is a prospective study. The study included 80 consecutive HCV-infected patients undergoing PEG IFN with ribavirin combination therapy (64 CHC and 16 LDLT patients) who attended the Nagasaki University Hospital for an initial visit between January 2005 and December 2007. All patients received the targeted dose of 1.5 μg/kg PEG IFN-α-2b (Pegintron; Schering-Prough K.K., Osaka, Japan) once weekly with daily ribavirin (Rebetol; Schering-Prough K.K., Osaka, Japan) for a total dose of 600 mg (bodyweight < 60 kg), 800 mg (60 kg < bodyweight < 80 kg) or 1000 mg (bodyweight > 80 kg) according to bodyweight (BW). The number of patients who were judged to have obtained a curative effect from IFN therapy was 42 in total, and 12 were LDLT patients. If the HCV-RNA had been negative in the patient serum until 12 weeks after the initiation of treatment or positive at 24 weeks, PEG IFN with ribavirin therapy was stopped at week 48. If the HCV-RNA had been negative from weeks 12 to 24, PEG IFN with ribavirin therapy was continued for 24 weeks to a predetermined 48 weeks. CHC patients were diagnosed on the basis of a persistently raised alanine aminotransferase (ALT) level and biopsy proven disease. All LDLT patients, who had undergone liver transplantation for HCV related cirrhosis at Nagasaki University Hospital from June 2002 to May 2007, had the HCV-RNA in their serum at the commencement of PEG IFN with ribavirin treatment. To prevent HCV related hepatitis after liver transplantation, pre-emptive therapy using IFN is the strategy used at the Nagasaki University Hospital. After the recovery of the general condition without ascites and icterus after transplantation, and establishment of the diagnosis using the liver biopsy, PEG IFN with ribavirin therapy was started. The interval between LDLT and IFN treatment was a mean of 281 days (range 16–989 days). Tacrolimus (Astellas, Tokyo, Japan), an immunosuppressive agent, was used together with steroids for all LDLT patients as the induction therapy. When IFN therapy was commenced, tacrolimus was switched to cyclosporin (Novartis, Tokyo, Japan) in 12/16 cases. A percutaneous liver biopsy assisted by ultrasonography was carried out in all cases. Liver histology was evaluated according to the degree of fibrosis and necroinflammatory activity.[13] The extent of fibrosis (staging) was classified as follows: F1 (periportal expansion), F2 (portoportal septa), F3 (portocentral linkage or bridging fibrosis) and F4 (cirrhosis). The necroinflammatory activity (grading) was classified as follows: A1 (mild), A2 (moderate) and A3 (severe). Liver biopsy specimens were fixed in 10% formalin, embedded in paraffin, cut to a thickness of 4 μm, and subjected to hematoxylin–eosin and Azan–Mallory staining.

Hepatitis C virus kinetics assessment

We compared the HCV viral load in both groups, determined by the HCV core antigen (cAg), at baseline (D0), day 1 (D1), week 1 (W1), week 2 (W2), week 4 (W4), week 8 (W8), week 12 (W12), week 24 (W24) and week 48 (W48). The HCV viral serotype (ST) and HCV cAg were determined using available kits. In this assay, HCV serotypes 1 and 2 correspond to genotypes 1 and 2 of Simmonds' classification,[14] respectively. The HCV cAg correlates with HCV-RNA by quantitative PCR.[15] HCV cAg was measured at the indicated times and HCV-RNA qualitative PCR, the amplicor monitor method, was used after the level was under the detection range of HCV cAg in every month. In the present study, we proposed the calculation of the decreased HCV viral load during PEG IFN with ribavirin treatment and set as follows: a negative HCV cAg was 20 fmol/L and a negative HCV-RNA qualitative PCR was 1 fmol/L.

Clinical and laboratory measurements

The body mass index (BMI) was calculated as weight (kg) divided by the square of height (m). Subjects fasted overnight before blood samples were obtained. Venous plasma glucose was measured with an automated analyzer, and basal serum insulin was measured using a standard radioimmunoassay. The index of insulin

resistance and β-cell function was calculated using the fasting value of plasma glucose (we excluded the patients with greater than 130 mg/dL), and the serum insulin level according to the homeostasis model assessment (HOMA) method. HOMA-IR, an insulin resistance marker, is calculated as follows: fasting plasma glucose × fasting insulin/405. HOMA-β, a β-cell function marker, was calculated as follows: 360 × fasting insulin/(fasting plasma glucose-63).[16] White blood cell, red blood cell, platelet, hemoglobin A1c, ALT, aspartate aminotransferase (AST), γ-GTP, total cholesterol (TC), triglyceride (TG), low density lipoprotein (LDL), high density lipoprotein (HDL), free fatty acid (FFA), and ferritin were determined by standard hematometry and laboratory techniques.

Statistical analysis

The data were processed on a personal computer and analyzed using StatView 5.0 (SAS Institute, Cary, NC, USA). Differences between groups were analyzed by Mann–Whitney *U*-test and Pearson χ^2-test. All data in the text and tables are shown as means, unless otherwise indicated. The statistical analysis of the HCV-RNA disappearance rate was by the Kaplan–Meier method with Wilcoxon assay. Values of $P < 0.05$ were considered to be statistically significant.

RESULTS

Differences of patient characteristics

FIRST, THE PRETREATMENT clinical and laboratory characteristics were compared with All-CHC and All-LDLT patients (Table 1). The BW and BMI in the All-CHC group were higher than that of the All-LDLT group. Therefore, the levels of PEG IFN dose per BW and ribavirin dose per BW were even, but the levels of PEG IFN dose and ribavirin dose in the all LDLT group were lower than in the All-CHC group. The HCV viral load in the all LDLT group was greater than that in the All-CHC group and serotype 1 was the majority in the All-LDLT group. In hematology and laboratory data, the red blood cell count and hemoglobin in the All-LDLT group was lower than that of the All-CHC group, and the FFA level was higher in the All-LDLT group. In the histological examination, fibrosis is more advanced in the All-CHC group than in the All-LDLT group. There was the tendency toward higher levels of fasting plasma glucose and lower levels of HOMA-β in the All-LDLT group than in the All-CHC group. Next, we targeted the serotype 1 and a high HCV titer (ST1H group) above 100 KIU/L by the qualitative PCR method or 300 fmol/L of the cAg assay. These were examined in the same way (Table 2). The ST1H group might have shown the same result as the All group, except the levels of fasting plasma glucose and HOMA-β did not differ with ST1H-CHC and ST1H-LDLT. The mean value of fasting plasma glucose (FPG) was higher than the normal range in the LDLT group. The discontinuance rates of treatment were almost equal, 19 cases (29.7%) and 4 cases (25%) in All-CHC and All-LDLT, respectively. The reasons for discontinuance were adverse effects in All-LDLT patients and the refractory nature of viral response in two All-CHC patients.

The HCV infection in the LDLT group is more obstinate than in the CHC group

The response rate and cure rate of PEG IFN with ribavirin therapy were compared with both groups (Table 2A, All group and B, ST1H group). The HCV response rate to treatment, viral response (VR), was determined by the disappearance of HCV-RNA or by the decline of HCV cAg to less than 1/100 before treatment. The cure rate, sustained viral response rate (SVR), was determined by a negative HCV-RNA by qualitative PCR method at 6 months post-termination of treatment. The VR rate at 8 and 12 weeks, but not at 4 weeks, and the PP-SVR in the LDLT group (Table 3A,B) was worse than that in the CHC group. Non-viral responders, who did not achieve HCV-RNA negativity during the treatment, did not show statistical significance in either SG1H group (Table 3B). As a result, we calculated the prediction of the lack of SVR by non-viral response in the LDLT group. The sensitivity, specificity, positive predictive values and negative predictive value were 1, 0, 0.917 and the acalculia for null viral responders at 24 h, 0.7, 1, 1 and 0.25 at 4 weeks, 0.6, 1, 1 and 0.2 at 8 weeks and 0.6, 1, 1 and 0.2 at 12 weeks, respectively.

The disappearance rate of HCV-RNA was evaluated by the Kaplan–Meier method (Fig. 1 ST1H group). The disappearance rate in the LDLT group was statistically lower than the CHC group. Before 14 weeks after the initiation of treatment, the HCV-RNA disappearance case was not apparent in the ST1H group (Fig. 1).

The decline of HCV load, especially early phase, is blocked in the LDLT group

For the analysis of viral kinetics, we evaluated the decline of the HCV load and the decline rate after treatment with particular emphasis of the early phase of treatment, including D1-W12. In the ST1H group (Fig. 2), the decreased rate on D1 in the LDLT group was

Table 1 Difference of characteristics between all chronic hepatitis C cases and all living donor liver transplantation cases

Characteristics	All-CHC ($n = 64$)	All-LDLT ($n = 16$)	*P*-value
Age (years)	58 ± 10.8	58.8 ± 4.62	NS
Sex (male : female)	36:28	7:9	NS
Height (m)	1.60 ± 0.098	1.583 ± 0.010	NS
Bodyweight (kg)	61.0 ± 11.0	54.8 ± 8.52	0.025
Body mass index	23.6 ± 2.94	21.8 ± 2.30	0.022
PEG IFN dose (μg)	80.1 ± 18.7	71.9 ± 33.5	0.035
PEG IFN/BW	1.31 ± 0.304	1.35 ± 0.708	NS
Ribavirin dose (mg)	621.9 ± 151.7	525 ± 100	0.030
Ribavirin/BW	10.2 ± 2.23	9.72 ± 2.04	NS
Serotype (1:2)	45:17	15:1	0.081
HCV cAg (fmol/L)	5773 ± 5609	23144 ± 21059	0.001
WBC (/μL)	5006.3 ± 1335	5918.8 ± 2439	NS
RBC (10^4/μL)	445 ± 41.1	350 ± 56.7	< 0.0001
Hemoglobin (g/dL)	13.8 ± 1.06	10.9 ± 1.85	< 0.0001
Platelet (10^4/μL)	16.4 ± 4.48	18.5 ± 10.6	NS
AST (U/L)	62.9 ± 35	64.3 ± 37.2	NS
ALT (U/L)	85 ± 53.0	89.9 ± 57.1	NS
γ-GTP (U/L)	62.1 ± 56.5	138.9 ± 129.1	0.013
Ferritin (ng/dL)	218 ± 216	254 ± 259	NS
TC (mg/dL)	169.8 ± 26.6	167.3 ± 38.8	NS
TG (mg/dL)	105.3 ± 46.8	122.8 ± 44.8	0.069
HDL (mg/dL)	45.2 ± 11.9	46.6 ± 14.9	NS
LDL (mg/dL)	97.3 ± 24.3	88.8 ± 26.7	NS
FFA (mEq/L)	0.492 ± 0.261	0.686 ± 0.299	0.019
FPG (mg/dL)	91.9 ± 15.4	125.1 ± 56.9	0.090
Insulin (mIU/L)	9.16 ± 5.1	8.34 ± 5.16	NS
HOMA-IR	2.08 ± 1.22	1.75 ± 1.42	NS
HOMA-β	135.4 ± 86.2	89.7 ± 86.9	0.075
Fibrosis	1.86 ± 1.18	0.875 ± 0.806	0.004
Activity	1.03 ± 0.48	1.31 ± 0.48	0.067

Data are shown as the means ± standard deviation and values, with statistical analysis calculated by Mann–Whitney *U*-test for means and Pearson's χ^2-test for values.
Normal values in laboratory tests: ALT (IU/L), 5–40; AST (IU/L), 10–40; γ-GTP (IU/L), < 70 in males, < 30 in females; TC (mg/dL), 150–219; TG (mg/dL), 50–149; FFA (mEq/L), 0.14–0.85; LDL (mg/dL), 70–139; HDL (mg/dL), 40–86 in male, 40–96 in female; hemoglobin (g/dL), 13.5–17.6 in male, 11.3–15.2 in female; WBC (/μL), 3900–9800 in males, 3500–9100 in females; RBC (10^4/μL), 427–570 in males, 376–500 in females; ferritin (mg/dL), 27–320 in males, 3.4–89 in females; platelet (10^4/μL), 13.1–36.2 in males, 13–36.9 in females; insulin (IU/L), 3.06–16.9; FPG (mg/L), 70–109. HOMA-IR, HOMA-β, and BMI are described in the text.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; FFA, free fatty acid; FPG, fasting plasma glucose; HCV cAg, hepatitis C virus core antigen; HDL, high density lipoprotein; HOMA, homeostasis model assessment; LDL, low density lipoprotein; LDLT, living donor liver transplantation; PEG IFN, pegylated interferon; RBC, red blood cell count; TC, total cholesterol; TG, triglyceride; WBC, white blood cell count.

statistically lower than CHC (Fig. 2b) and the viral load of the LDLT group was larger than that in CHC from D0 to W12 (Fig. 2a). The decreased rate at the indicated time without D1 and W12 was not the difference between CHC and LDLT (Fig. 2b). We next analyzed the SG1H-group that matched the pre-treatment HCV cAg titer (Fig. 3). In a similar fashion to Figure 2, the viral load of the matched LDLT group was larger than that of the matched CHC from D1 to W12 (Fig. 3a) and the decreased rate of the matched LDLT group was lower than that of the matched CHC at D1, W2 and W4 (Fig. 3b).

DISCUSSION

IN THE PRESENT prospective study, we compared CHC and LDLT patients treated with PEG IFN and ribavirin for HCV infection. BMI, HCV cAg, red blood

Table 2 Difference of characteristics of serotype 1 and high virus titer between chronic hepatitis C patients and living donor liver transplantation patients

Characteristics	ST1H-CHC ($n = 42$)	ST1H-LDLT ($n = 15$)	*P*-value
Age (years)	58.5 ± 10.8	58.8 ± 4.78	NS
Sex (male : female)	22:20	6:9	NS
Height (m)	1.60 ± 0.10	1.566 ± 0.081	NS
Bodyweight (kg)	61.8 ± 12.1	53.8 ± 7.69	0.02
Body mass index	24.0 ± 2.78	21.9 ± 2.37	0.012
PEG IFN dose (μg)	81.4 ± 19.5	73.3 ± 34.2	0.052
PEG IFN/BW	1.33 ± 0.269	1.39 ± 0.711	NS
Ribavirin dose (mg)	642.8 ± 150.0	520 ± 101.4	0.011
Ribavirin/BW	10.5 ± 2.13	9.80 ± 2.08	NS
HCV cAg (fmol/L)	6969 ± 5281	24674 ± 20856	0.003
WBC (/μL)	5019.0 ± 1294	6033.8 ± 2479	NS
RBC (10^4/μL)	444 ± 40.1	351 ± 58.6	< 0.0001
Hemoglobin (g/dL)	13.9 ± 1.10	10.8 ± 1.88	< 0.0001
Platelet (10^4/μL)	16.7 ± 4.68	18.9 ± 10.8	NS
AST (U/L)	62.1 ± 31.6	64.2 ± 38.5	NS
ALT (U/L)	84.5 ± 51.8	88.0 ± 58.6	NS
γ-GTP (U/L)	64.0 ± 61.7	113.6 ± 83.1	0.036
Ferritin (ng/dL)	206 ± 164.8	204.5 ± 188.4	NS
TC (mg/dL)	172.6 ± 25.7	165.3 ± 39.2	NS
TG (mg/dL)	108.2 ± 52.2	122.9 ± 46.4	NS
HDL (mg/dL)	46.5 ± 11.9	45.4 ± 14.8	NS
LDL (mg/dL)	97.7 ± 25.4	88.6 ± 27.8	NS
FFA (mEq/L)	0.514 ± 0.251	0.693 ± 0.310	0.049
FPG (mg/dL)	92.4 ± 16.4	123.7 ± 58.6	NS
Insulin (mIU/L)	9.06 ± 5.5	8.34 ± 5.16	NS
HOMA-IR	2.07 ± 1.31	1.86 ± 1.38	NS
HOMA-b	128.0 ± 76.2	95.7 ± 86.5	NS
Fibrosis	1.92 ± 1.19	0.933 ± 0.799	0.008
Activity	1.08 ± 0.474	1.33 ± 0.488	0.098

Data are shown as the means ± standard deviation and values, with statistical analysis calculated by Mann–Whitney *U*-test for means and Pearson's χ^2-test for values.
Normal values in laboratory tests are same as in Table 1.
ALT, alanine aminotransferase; AST, aspartate aminotransferase; CHC, chronic hepatitis C; FFA, free fatty acid; FPG, fasting plasma glucose; HCV cAg, hepatitis C virus core antigen; HDL, high density lipoprotein; HOMA, homeostasis model assessment; LDL, low density lipoprotein; LDLT, living donor liver transplantation; PEG IFN, pegylated interferon; RBC, red blood cell count; TC, total cholesterol; TG, triglyceride; WBC, white blood cell count.

cell, γ-GTP, FFA and liver fibrosis in the pretreatment clinical characteristics were different in both groups (Tables 1,2). The VR rate of the CHC group was superior to that of the LDLT group, and the SVR by per-protocol analysis was also similar in result to the VR (Table 3). The viral disappearance rate of the CHC group was superior to the LDLT group, regardless of the HCV serotype (Fig. 1). The HCV cAg titer under the treatment in the LDLT group was more than that of the CHC group from D0 to W12 (Figs 2a,3a) and the HCV cAg decrease rate of the LDLT group at the D1 was less than that of the CHC group (Figs 2b,3b). We showed that the reinfected HCV to the graft liver was more refractory than the non-transplanted CHC. The PEG IFN and ribavirin dose per BW was an equal dose in both groups. However, it was difficult to determine the pretreatment predictive factors for the LDLT cases, because only one case showed SVR in the LDLT group. Thus, we considered that the difference of the pretreatment clinical characteristics in both groups might be related to the refractory HCV infection.

The pretreated HCV cAg titer is known to be the principal factor for IFN resistance. For CHC and LDLT patients, a high HCV-RNA titer in the pretreatment sera

Table 3 Result of pegylated interferon-α-2b plus ribavirin therapy

A. All cases			
Term	All-CHC	All-LDLT	*P*-value
Viral response 4 weeks	40/60 (67%)	5/12 (42%)	NS
Viral response 8 weeks	47/55 (85%)	6/12 (50%)	0.011
Viral response 12 weeks	43/48 (90%)	6/12 (50%)	0.003
Sustained viral response: ITT	20/42 (45%)	2/12 (20%)	0.054
Sustained viral response: PP	20/28 (80%)	2/9 (22%)	0.008
B. Serotype 1 and high virus titer cases			
Term	ST1H-CHC	ST1H-LDLT	*P*-value
Viral response 4 weeks	24/40 (67%)	5/11 (45%)	NS
Viral response 8 weeks	30/36 (83%)	5/11 (45%)	0.012
Viral response 12 weeks	25/29 (86%)	5/11 (45%)	0.008
Sustained viral response: ITT	8/27 (30%)	1/11 (8%)	NS
Sustained viral response: PP	8/15 (53%)	1/9 (11%)	0.029
Non-virological response: ITT	11/27 (41%)	5/11 (45%)	NS
Non-virological response: PP	4/15 (27%)	4/9 (44%)	NS

Data are shown as relevant numbers/target case numbers (percentage of relevant numbers) with statistical analysis using Pearson's χ^2-test for numbers.
CHC, chronic hepatitis C; ITT, intention to treatment analysis; LDLT, living donor liver transplantation; PP, per-protocol analysis.

is associated with non-responder status for IFN treatment.[7,17] In the LDLT condition, the HCV-RNA titer was rapidly increased after immediately decreasing at transplant and the viral load after several weeks post-LDLT exceeded the value of pre-LDLT.[18] The HCV-RNA titer increased rapidly in patients receiving corticosteroids as part of the immunosuppressant regimen.[18,19] We have speculated that the massive amount of HCV, caused by immunosuppressant therapy after the LDLT, was part of the reason for the IFN refractory status. However, comparisons with the pretreated HCV cAg matched groups (Fig. 3) showed the existence of an important factor other than the pretreatment viral load. It will, therefore, be necessary to analyze this problem by evaluating many factors, for example immunosuppressants[10] and regeneration, in the future.

A high level of γ-GTP was also known to be an important factor for IFN treatment.[7,17] Usually, high levels of γ-GTP and FFA have been linked to insulin resistance.[20,21] Therefore, insulin resistance in the liver is assumed in the condition of IFN resistance. However, the LDLT group had the normal range of HOMA-IR,[16] which was lower than that of the CHC group (Tables 1,2). The HCV infection after liver transplantation is associated with insulin resistance.[22] Immunosuppressants, especially corticosteroids, induced insulin resistance.[23] In the present study, the LDLT group had a disturbance of insulin secretion

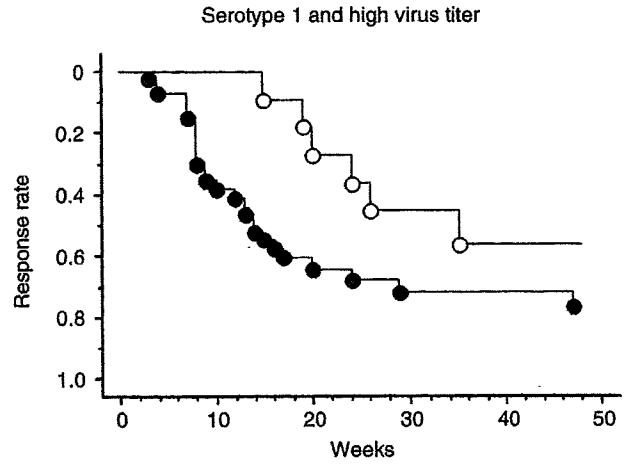

Figure 1 The difference in the hepatitis C ribonucleic acid (HCV-RNA) disappearance rate between the chronic hepatitis C (CHC) group and living donated liver transplantation (LDLT) group during 48 weeks of treatment. HCV-RNA was evaluated by the qualitative PCR method. The disappearance rate was calculated as follows: serum HCV-RNA disappearance case number/all cases in indicated time. The statistical analysis was carried out using the Kaplan–Meier method with the Wilcoxon assay. ST1H group was plotted as the HCV-RNA disappearance line between the white circle of the LDLT group and the black circle of the CHC group. In all cases and the ST1H group, the disappearance rate was statistically significant between the CHC group and the LDLT group ($P < 0.05$).

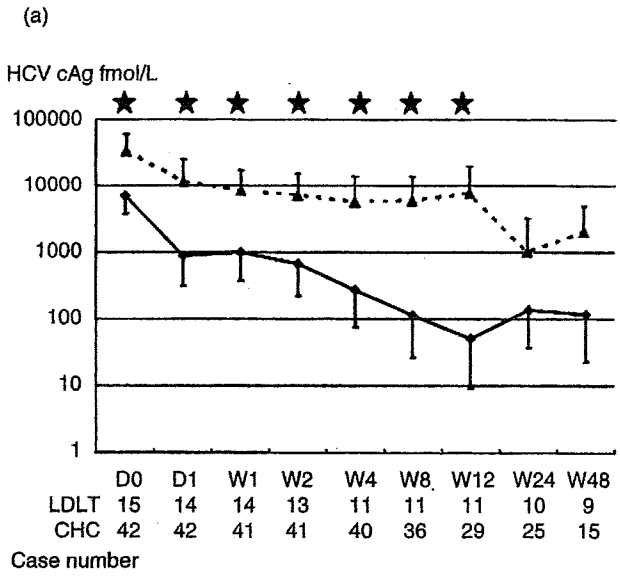

Figure 2 Comparison of viral kinetics between the SG1H-chronic hepatitis C (CHC) group and the SG1H-living donor liver transplantation (LDLT) group during the 48 weeks of treatment. (a) The hepatitis C virus core antigen (HCV cAg) load and (b) reduction rates were plotted by a straight line (SG1H-CHC group), and dotted line (SG1H-LDLT group). The error bar represented the standard deviation. On the *y*-axis, D0 is pretreatment, D1 and WX is time post-treatment day 1 and week X, respectively. The reduction rate was calculated as follows: $\log_{10}$HCV cAg load in indicated time/in D0. HCV cAg titer at the indicated time between SG1H-CHC and SG1H-LDLT were compared. The asterisk mark indicates a significant difference, $P < 0.05$, calculated by Mann–Whitney *U*-test.

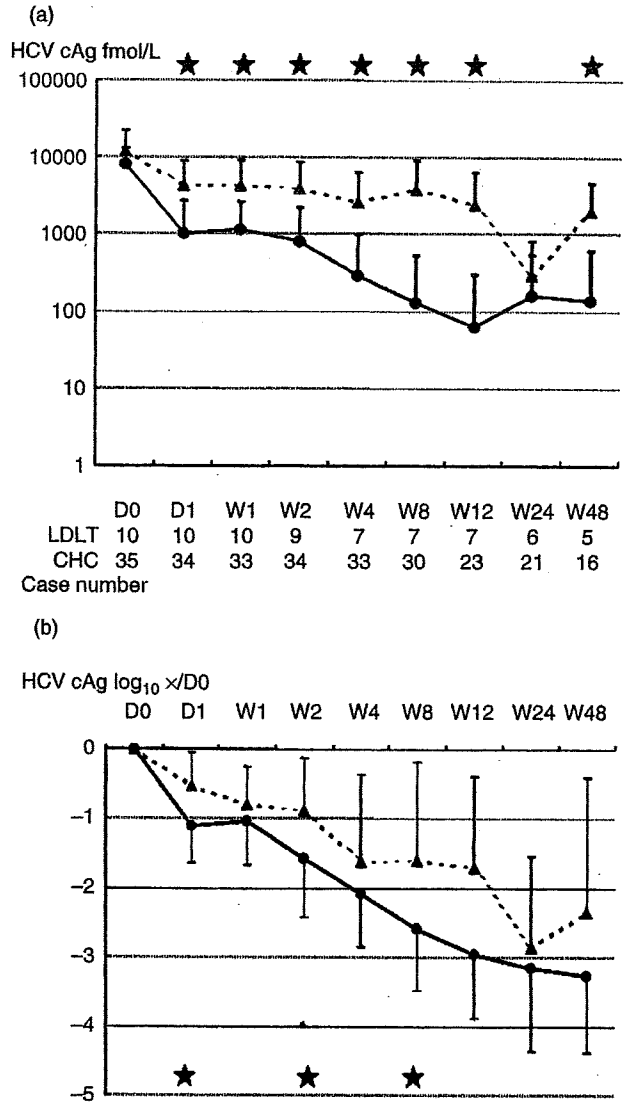

Figure 3 Comparison of viral kinetics between matched pre-treatment hepatitis C virus core antigen (HCV cAg) ST1H-chronic hepatitis C (CHC) group and ST1H- living donor liver transplantation (LDLT) group during 48 weeks of treatment. (a) HCV cAg load and (b) reduction rate were plotted by a straight line (matched SG1H-CHC group) and dotted line (matched SG1H-LDLT group). The error bar represents the standard deviation. The asterisk mark is the significant difference, $P < 0.05$, calculated by Mann–Whitney *U*-test.

rather than insulin resistance and high levels of FPG might be caused by the disturbance of insulin secretion. Therefore, further study is necessary to clarify the relationship between the glucose metabolism and the IFN resistance in LDLT patients. The levels of γ-GTP rise at cholestatic conditions. It was reported that the presence

of a cholestatic profile is associated with an adverse response to IFN treatment in LT.[7] A cholestatic profile provoked the TH2-like lymphocyte response.[19] The authors have previously reported that IL-10, representative of TH2 cytokine, inhibits IFN signaling through an inducible suppressor of cytokine signaling.[24] The high levels of FFA were induced by a catabolic state, such as cirrhosis, and were not fully recovered after LDLT. As a result, the levels of FFA reflected a continuous catabolic state at the beginning of IFN treatment. FFA can induce oxidative stress in various cells,[25,26] and inhibit the IFN induced antiviral gene induction through the inactivation of Jak-1 and Tyk-2.[27] Therefore, we are speculating that high levels of γ-GTP and FFA in the LDLT group have the ability to inhibit IFN signaling as much as in the CHC patients.

We are paying attention to the viral decline of D1/D0 (Figs 2b,3b). The decreased rate of D1 is named as the first phase of HCV decline and is the predictor of SVR.[28,29] The first phase influenced the second phase, which is the decline of HCV after D2.[28] The IFN induced antiviral gene products were considered to be very important for antiviral activity.[11] The expressions of the IFN stimulating genes (ISG) were associated with the early phase of the decline[11] and it was reported that the lack of ISG caused early liver fibrosis in the LT patients with HCV.[30] In the LDLT group, the reduced HCV cAg decreased the rate of D1 and this might be part of the cause of being refractory to IFN. We speculate that an IFN signaling disturbance, related to high levels of γ-GTP and FFA, might have triggered the adverse effect to the HCV cAg decreased rate of D1.

In summary, it became clear that the viral response and SVR is worse in the LDLT group. The first phase of viral decay, the decreased rate of D1/D0, also declined in the LDLT group. High levels of γ-GTP and FFA in the pretreatment sera might also be related to IFN-signaling damage in hepatocytes. At the initiation of pre-emptive therapy, HCV had also been increasing in the graft liver and the catabolic status of energy did not recover for the relatively small size of the graft liver. When beginning treatment for an HCV infection after LT, we should carefully take into account the timing of IFN initiation, in addition to the types of immunosuppressants used.

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Indocyanine Green Dye Excretion in Bile Reflects Graft Function After Living Donor Liver Transplantation

There are many biochemical parameters that can be used to assess the function of partial liver graft; however, a definitive marker remains to be identified. For example, the color of bile has been reported anecdotally to reflect graft function, but its value has not yet been fully evaluated. Recently, indocyanine green (ICG) K value, that is ICG clearance, was found to be valuable in assessing the function of a whole or partial graft after living donor liver transplantation (LDLT) (*1*). However, the measurement of ICG dye concentrations in serum is not valid in hyperbilirubinemic patients because of interference while using the spectrophotometer (*2*). We previously reported the value of ICG dye concentration in the bile in an ICG challenge test in patients undergoing liver resection for bile duct cancer, because ICG is a relatively hydrophobic organic anion that has a high hepatic extraction ratio and is excreted extensively and remains unchanged in bile (*3*). It has also been reported that ICG excretion in bile could be a good parameter for assessing preoperative liver function in biliary drainage patients because it reflects adenosine triphosphate levels in the liver (*4, 5*). Thus, this study evaluates ICG excretion in bile after LDLT as a potential novel parameter of partial liver graft function. This prospective study was approved by the local institutional review board, and written informed consent was obtained from all patients.

Of 37 consecutive patients who underwent liver transplantation between April 2005 and March 2007, 32 patients who received duct-to-duct biliary reconstruction were studied (median age 53 years; right lobe graft:left-sided graft 19:13; and graft volume/standard liver volume median 46%). One week after LDLT, we performed a fasting ICG injection test (0.5 mg/kg; Diagnogreen Inj.; Daiichi Seiyaku, Tokyo, Japan), and bile was collected through biliary external stenting reported earlier (*6*) while blocking natural light. ICG concentrations in bile at 0, 30, 120, and 360 min after injection were measured by spectrophotometer at a wavelength of 805 nm. Blood samples were also taken before ICG injection and 15 min after injection. All data were expressed as median values with ranges.

The pattern of change in ICG excretion after ICG injection showed distinct two patterns. Type I (n=24) showed a steep increase and a peak within 2 hr and returned to baseline within 6 hr, whereas type II (n=8) showed a low peak of less than 1 micromole per liter during the 6-hr observation period. Table 1 shows various detailed parameters of type I and type II patients and their LDLTs. The parameters that correlated with ICG patterns were compared between the two groups, and donor age and graft type were found to have a significant influence on the type of ICG excretion in bile. Also, it shows the relationship between the type of ICG excretion in bile and various graft functions such as jaundice, prothrombin activity, and ICG retention rate at 15 min. In fact, because retention rate of ICG at 15 min after injection was correlated with type of ICG excretion in bile, ICG retention rate at 15 min can substitute the excretion pattern in bile. Several type II patients had prolonged jaundice after LDLT due to outflow block (n=1), acute cellular rejection (n=2), or unknown cause (n=5).

To clarify changes in ICG pattern over time, the same challenge test was performed at 3 months after LDLT. Most patients remained or became type I (data not shown). Six of the eight patients who were classified as type II at 1 week after LDLT were classified as type I at 3 months after LDLT. Remaining two patients had prolonged jaundice until approximately 6 months after LDLT and recovered without jaundice. These two patients were complicated with biliary

TABLE 1. Factors influencing types of ICG excretion in bile and various outcomes after LDLT

	Type I (n=24)	Type II (n=8)	*P*
Recipient age (yr)	56 (31–68)	61(16–68)	0.3005
Gender (male:female)	17:7	4:4	0.4336
Graft (right lobe:right posterior sector:left lobe)	13:1:10	2:3:3	0.0273
GV/SLV (%)	43.4 (24–65)	43 (26–65)	0.8748
Donor age (yr)	32 (20–58)	48 (20–61)	0.0348
ABO incompatible	4 (17%)	2 (25%)	0.3472
Acute cellular rejection	2 (8%)	2 (25%)	0.1840
Warm ischemic time (min)	43 (30–59)	42 (31–61)	0.7012
Cold ischemic time (min)	111 (50–236)	121 (60–192)	0.6321
Maximal T. Bil (mg/dL)	5.1 (1.2–33.6)	12.9 (3.9–30.2)	0.0394
POD of maximal T. Bil	2.5 (1–28)	13.5 (1–28)	0.0599
POD when T. Bil less than 5 mg/dL	5 (0–80)	27.5 (1–118)	0.0179
Minimal PT (%)	29 (19–42)	29.5 (22–50)	0.8190
ICG R15 (%)	10 (1–77)	31 (11–70)	0.0172
In-hospital death	1	0	0.5657

Data are expressed as median (range).
ICG, indocyanine green; LDLT, living donor liver transplantation; GV/SLV, graft volume per standard liver volume of the recipient; T. Bil, total bilirubin; POD, postoperative days; PT, prothrombin time.

stricture and chronic rejection at 3 months after LDLT.

Donor age has been reported to be an important factor of graft quality and liver regeneration (*7*). In addition, we demonstrate in this study that not only donor age but also graft type affects the bile excretion of the graft liver. Right posterior sector grafts are sometimes problematic because the portal vein is smaller at the second-grade bifurcation, causing relative portal hypertension and biliary stricture (*8*)

Type II pattern at 1 week after LDLT is not a predictor of poor outcome with a partial graft, although prolonged jaundice should be expected. In fact, duration until the disappearance of icterus is of great interest after liver transplantation. This study also demonstrates that ICG excretion in bile is a direct indicator of prolonged severe jaundice.

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The Use of Inferior Epigastric Artery in Renal Transplantation

Transplantation of donor kidneys with multiple arteries, especially when one is 1 mm or less, may lead to technical problems. This is especially pertinent with short lower pole arteries that supply the ureter and should not be ligated. We report the advantage of an uncommonly used sequential revascularization of the lower (or upper) pole of the donor kidney and ureter using the recipient's inferior epigastric artery, a branch of the external iliac artery, after the kidney is revascularized through the main artery.

We, retrospectively, reviewed the long-term outcomes of renal allografts in which small donor lower or upper polar arteries (~1 mm) were anastomosed to the recipients' inferior epigastric arteries, which were initially dissected and prepared for that purpose. Between 2003 and 2006, seven patients received living (5) or deceased (2) donor kidneys with 1-mm lower pole (5) or upper pole (2) artery transplanted by one surgeon (M.A.H.). Immunosuppression included induction with antilymphocyte immunoglobulin, tacrolimus (6) or sirolimus (1) and MMF. Good perfusion by Duplex Ultrasonography of the graft, including the small polar artery, was present in all cases with follow-up studies of 4 months to 4 years, with six patients imaged more than 1 year after operation. No further nuclear imaging procedures were performed, but the Duplex USG follow-up at 1 to 5 years showed uniform renal perfusion without defects. In seven patients, the creatinine levels decreased rapidly; one graft, however, was lost after 1 year, whereas the others continued to function between 1 and 5 years, with serum creatinine ranging from 1.1 to 3.5 (eGFR of 37.6 cc/min in this patient).

There were no ureteral problems or parenchyma infarcts noted although two patients had transient moderate hydronephrosis immediately after transplantation, which resolved before discharge. The use of the inferior epigastric artery as a source of arterial inflow to the kidney was first described by Dubernard et al. in 1976 (*1*) as an alternative to standard donor-recipient arterial anastomoses. A recent report by El-Sherbiny et al. (*2*) reemphasized the selection of arterial revascularization of kidneys with multiple arteries using inferior epigastric artery. Wolters et al. (*3*) described that ureteral necrosis after renal transplantation may result from impaired perfusion because of loss of blood supply from the donor's lower polar arteries and suggested the option of using the inferior epigastric artery to avoid such complications. Other authors, Kumar et al. (*4*) used the same arteries and found decreased rewarm ischemia time and reduced incidence of acute tubular necrosis. The use of the recipient inferior epigastric artery as an arterial supply for the donor lower pole artery was also previously documented by Young et al. (*5*) as providing an excellent flow to the lower pole of the kidney. The downside is that this type of anastomosis should be reserved for renal vessels that are shorter than 2 to 3 cm and smaller than 2 to 3 mm. The procedure also requires excellence in microvascular surgery with avoidance of kinking of the vessels.

We conclude that the inferior epigastric anastomosis to a small polar vessel in a multiple artery kidney can be successfully used in renal transplanta-

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ORIGINAL ARTICLE—LIVER, PANCREAS, AND BILIARY TRACT

Actual therapeutic efficacy of pre-transplant treatment on hepatocellular carcinoma and its impact on survival after salvage living donor liver transplantation

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Abstract
Background The exact efficacy of pre-liver transplant (LT) therapy for hepatocellular carcinoma (HCC) and the impact on survival after LT remain controversial in regard to salvage LT.
Materials and methods Of 79 patients transplanted in Nagasaki University Hospital between August 1997 and December 2007, 29 patients (36.7%) were indicated for HCC based on the Milan criteria using computed tomography and magnetic resonance imaging. Pre-LT therapy other than liver resection had been performed in 18 cases (62.1%) for 24 lesions. Treated lesions were analyzed histologically using thin slices of the whole explanted liver.
Results Pre-LT therapy included transarterial chemoembolization (TACE) for 10 lesions, percutaneous ethanol injection (PEI) + TACE for 1 lesion, PEI in 6 lesions and ablation therapy in 7 lesions. Under preoperative imaging study, 19 lesions (79.1%) were "thought-to-be" necrotic by pre-LT therapy. However, histologically, viable HCCs were still observed in 9 lesions (9/19 47%). A median interval between the first pre-therapy and LT was 22 months, while last pre-LT therapy and LT was 11 months. No sarcomatous HCC or forced portal venous tumor thrombus was found in all cases with residual lesions. One peritoneal recurrence has occurred after LT, in whom PEI and RFA had been performed before LDLT. The disease free survival after LDLT was comparable to that of cases without pre-LT therapy.
Conclusion Half of the preoperatively "thought-to-be" necrotic lesions still contained viable HCC cells after the pre-LT treatment. Overall, the history of pre-LT therapy does not preclude or interfere with subsequent LT, although percutaneous treatment may spread disseminated tumor cell growth under immunosuppression.

Keywords HCC · pre-LT · Recurrence

Introduction

In Japan, where the availability of deceased liver donors is limited, hepatocellular carcinoma (HCC) is primarily treated with hepatic resection, locoregional therapy and transarterial chemoembolization [1–3]. However, when HCC recurs and further treatment is no longer possible, liver transplantation (LT) may be considered as salvage LT [4].

There are drawbacks of pre-LT treatment for HCC during the waiting period. Dissemination [5] and implantation [6] may occur after puncture of HCC and they may form tumors after the administration of immunosuppressive drugs. In addition, after incomplete locoregional therapy, sarcomatous changes have been reported [7]. With subsequent liver transplantation, damage to vital vascular structures can occur (hepatic artery, portal vein) which may affect the outcome of liver transplantation. Therefore, pre-LT therapy for HCC may increase the possibility of unfavorable changes in HCC and mask the possibility of occult HCC in a background liver, thereby compromising the

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outcome of LT. Therefore, the effect of pre-LT therapy on HCC and the outcome of LT for HCC, especially within the Milan criteria, have been reported with mixed results [8–15].

A recent paper has reported the multiple occurrence and spread of HCC in a cirrhotic liver using whole liver histological examination (WLHE). However, the exact therapeutic effect of pre-LT treatment on primary HCC has not been analyzed. Explant analyses using WLHE were used, since it is the only opportunity to investigate the true viability of HCC in thought-to-be completely necrotic lesions following pre-LT therapy in imaging. This study investigates the accuracy of pre-LT therapy for HCC and its impact on the outcome of LT.

Materials and methods

Patients

Of 79 cases transplanted in Nagasaki University Hospital before Dec. 2007, 29 cases (36.7%) were indicated for HCC within the Milan criteria and for 18 cases (62.1%), pre-LT therapy other than liver resection was performed (Fig. 1). WLHE was performed by dedicated pathologists, with 5–7 mm slices for whole liver explants. Residual HCCs after pre-LT therapy were investigated histologically in combination with various factors.

This study was approved by the local Institutional Review Board and written informed consent was obtained from all patients.

Patient characteristics

All patients were indicated for LDLT as "salvage LT". The etiology in these cases was hepatitis C virus (HCV) infection in 12 patients and hepatitis B virus (HBV) in 6 patients. There were 7 females and 11 males, with a median age of 57 years (range, 48–61 years). The median values of alpha-fetoprotein (AFP) and protein-induced vitamin K antagonists II (PIVKA-II) were 30.25 ng/ml (range 0.8–806.1) and 23 g/ml (range, 6–247).

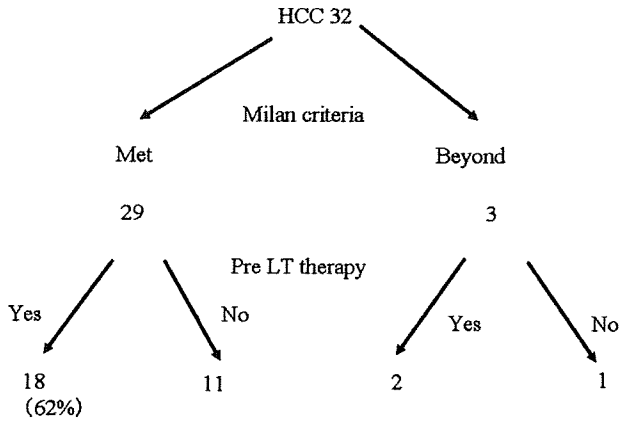

Fig. 1 Patient demographics. *LT* liver transplantation, *HCC* hepatocellular carcinoma

Liver transplantation and preoperative therapy for HCC

The median follow-up period was 24 months (range, 12–45 months). Pretreatment for HCC was performed prior to LT for 24 lesions in 18 patients, which included transarterial chemolipiodolization (TACE) in 10 lesions, radiofrequency ablation (RFA) in 7 lesions, percutaneous ethanol injection therapy (PEIT) in 6 lesions, TACE with PEIT in 1 lesion. Based on the imaging findings, all HCCs were considered to be within the Milan criteria. The clinical characteristics of the 18 patients are summarized in Table 1.

All lesions were surveyed by multidetector computed tomography scanning (MDCT) and magnetic resonance imaging (MRI) done within 1 month before transplant. Preoperatively "thought-to-be" necrotic lesions were not counted as HCC lesions under the Milan criteria. Only "thought-to-be" viable lesions evaluated by MD-CT with contrast media and MRI-SPIO were counted, which is also in accord with the Japanese national health insurance system. Preoperative imaging findings showed 5 patients with solitary viable HCC, 5 patients with double viable HCCs, 1 patient with triple viable HCCs and 7 patients with no viable HCCs. All patients met the Milan criteria with a solitary nodule 5 cm in size or 3 nodules 3 cm in size for multi-nodular HCC [18].

Whole liver histological examination (WLHE) [16]

After explantation, the cirrhotic livers were fixed in formalin for 48 h. The livers were then sectioned at

Table 1 Details in 18 patients receiving pre-LT therapy

TACE	10
Ethanol injection	6
Ablation	7
TACE + ethanol injection	1
Size of treated HCC	18 mm (10–30)
Number of therapy	2 (1–4)
Period between 1st therapy and LDLT	22 months (3–58)
Period between last therapy and LDLT	11 months (3–58)

TACE transarterial chemoembolization, *RFA* radiofrequency ablation, *HCC* hepatocellular carcinoma, *LDLT* living donor liver transplantation

5–7 mm intervals and each section was carefully inspected and mapped. All sections were embedded in paraffin and all slides were made from the paraffin-embedded material and routinely stained with hema-to_xylin and eosin. The median total number of slides for each patient was 116.5 (range, 64–185 slides). All slides were examined by an experienced pathologist (co-authors S.O. and H.M.). The pathological diagnoses and analyses were made according to the fourth edition of *The General Rules for the Clinical and Pathological Study of Primary Liver Cancer*, published by the Liver Cancer Study Group of Japan [17].

Statistical analysis

A statistical comparison of survival after LT was performed using the Kaplan-Meier method and compared by the log-rank test. Results were considered statistically significant when the P values were less than 0.05.

Results

At the time of LDLT, there was no sign of extrahepatic cancer spread in any of the patients. Histologically, after pre-LT therapy, viable HCCs were still observed in 9 (47%) out of 19 "thought-to-be necrotic" lesions (Fig. 2). The median period between pre-therapy and LT was 22 months (range 3–58 months). In 3 lesions, residual HCCs were found in the area next to necrotic area (Fig. 3), while in 6 lesions, residual HCCs were found within the same nodule (Fig. 4). With regard to tumor differentiation, there ... sarcomato... HCC in the treated residual lesions. No "forced" portal venous tumor thrombus was found around the remaining viable HCCs.

After LDLT, no recurrence was found except for one peritoneal dissemination (1/18:5.5%) after OLT. Since the type of recurrence is unusual after OLT, this case report will be shown below.

In fact, there were three patients transplanted for HCC beyond Milan Criteria, two of which had undergone pre-LT therapy (Fig. 1). In one patient, HCC recurred in the lung

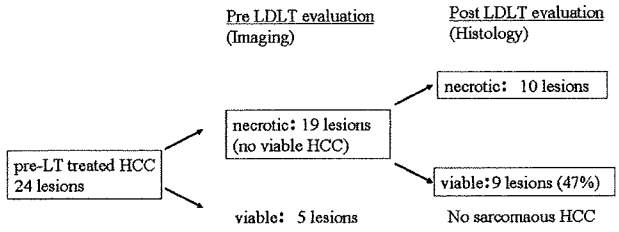

Fig. 2 Actual effect of pre-LT therapy on HCC. *LDLT* living donor liver transplantation, *HCC* hepatocellular carcinoma

12 months after LDLT, and he underwent partial lung resection three times. As pre-LT treatment, he had undergone liver resection and multiple TACE before LDLT. Another patient died at one month after LDLT due to hemophagocytic syndrome.

Survival after LDLT

After LT, regardless of pre-LT therapy, patient survival and recurrence-free survival were comparable between the groups (Fig. 5)

Case report

One patient underwent pre-LT treatments and subsequently developed peritoneal recurrence after LDLT. The patient was a 62-year-old male who had suffered from end-stage liver cirrhosis due to hepatitis C virus infection and was indicated for LDLT in May, 2006. Previously, he underwent a caudate lobe resection of the liver for HCC in 2001 and TACE for HCC in segments 4 and 7 in 2003. Subsequently, he was treated with TACE for HCC in segments 4 and 7 in 2004 and PEIT for HCC in segment 7 in 2004. RFA was a procedure of choice for an HCC in segment 2 in January, 2006. Finally, he developed end-stage liver failure and underwent a transplant in May, 2006. In the explanted liver, under WLHE, no viable HCCs were found.

However, following an increase in the AFP and PIVKA-II, two mass lesions were found in Douglas's pouch and the left lower abdomen in October, 2007. He had been on cyclosporine monotherapy as immunosuppression. Two lumpectomies were performed, which revealed moderately differentiated HCC under histological examination. It was presumed that the pre-LT treatment had disseminated the HCC, which developed slowly after the LDLT (Fig. 6). In August 2008, the patient died due to the multiple recurrence of HCC (local recurrence in the Douglas's pouch, bone metastasis, multiple liver metastases in the graft and multiple lung metastases).

Discussion

The present study demonstrated that after pre-LT therapy, 47% of the lesions still had viable HCC cells. Previously, Kim et al. [19] reported that a viable tumor volume ratio greater than 10% after pre-LT therapy was a significant prognostic factor. Pompili et al. [20] also reported that 58.7% of HCC had partial necrosis after percutaneous ablation procedures and the effect depended on the size of HCCs. Also, Wong et al. reported that fifteen nodules in five patients had <75% necrosis and these were due to

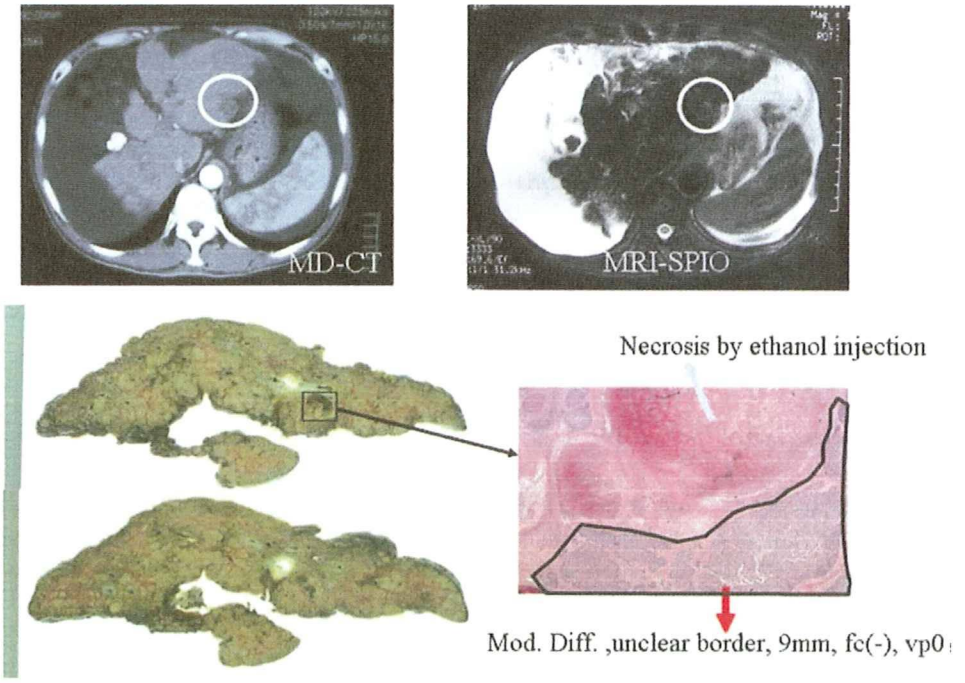

Fig. 3 A case presentation of a 58-year-old male. Liver cirrhosis due to hepatitis B viral infection, pre-LT imaging diagnosis: HCC 0

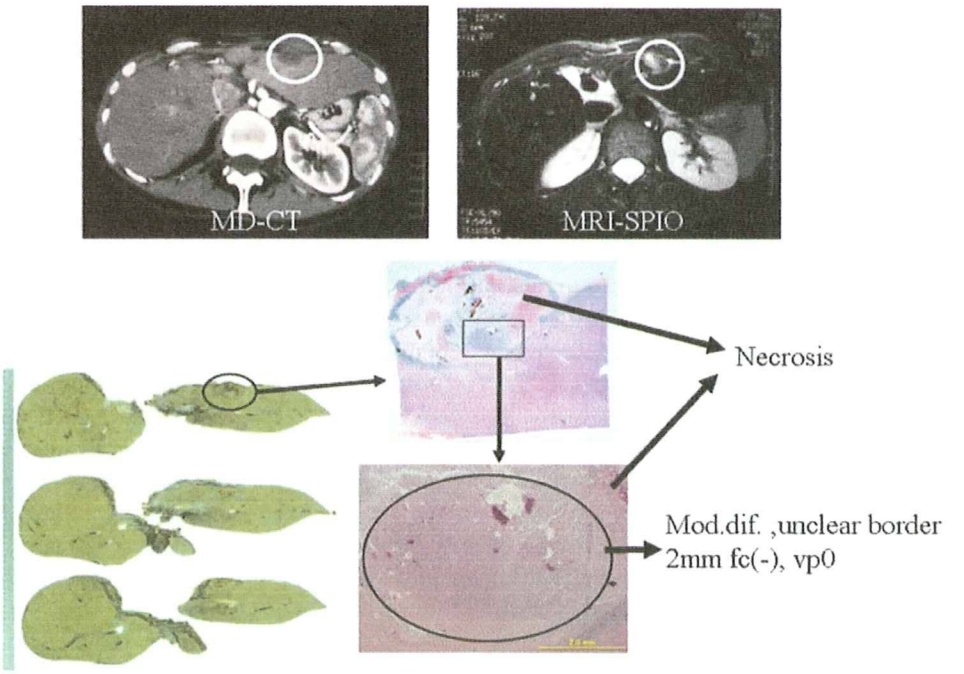

Fig. 4 A case presentation of a 33-year-old female. Liver cirrhosis due to hepatitis B viral infection, pre-LT imaging diagnosis: HCC 0

local/non-local recurrences or perhaps suboptimal treatment with RFA, TACE or cisplatin gel injection [21]. The mean waiting time for LT was 162.5 days. Nine of 13 patients had a different number of nodules than before pre-LT therapy, although stage changed in only three patients. The last pre-LT therapy and LDLT, which was median 11 months in our study, signified that salvage LDLT was considered and performed with 1 year for HCC bearing patients with viral hepatitis. Indeed, the outcome of LDLT even after at most pre-LT therapy, showed good disease-free-survival.

However, in the current study cohort, one case had peritoneal metastasis although PEI and RFA had been performed before LDLT. The patient finally died 2 years after LDLT with systemic metastases following resection

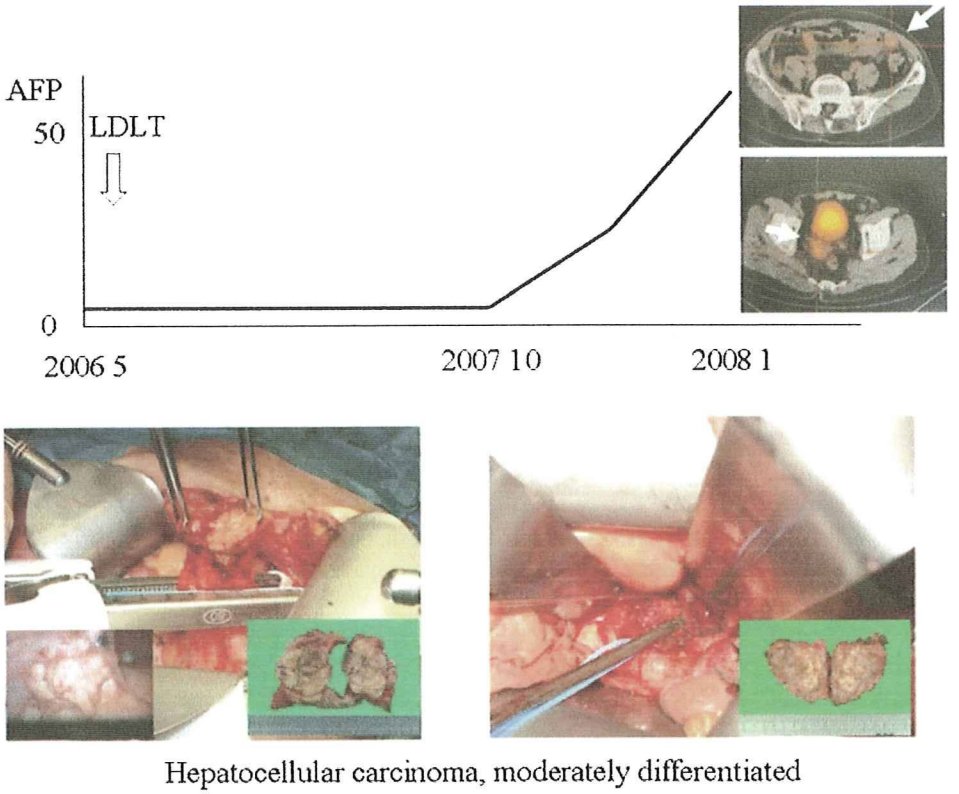

Fig. 5 A case presentation of peritoneal dissemination after LDLT. *LDLT* living donor liver transplantation

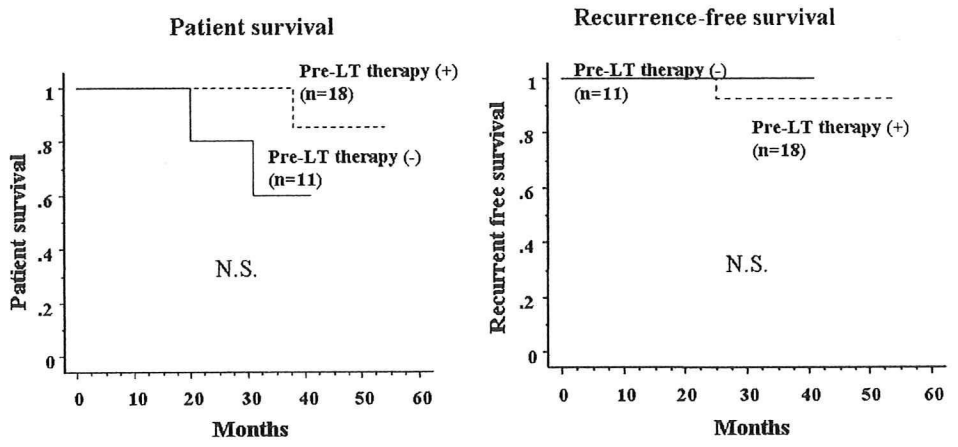

Fig. 6 Patient survival and recurrence-free survival after liver transplantation for patients with HCC within the Milan criteria

of 2 peritoneal metastases. In this case, there was no residual HCC in the explanted liver at the time of LDLT. This case illustrates a worst-case scenario with regard to pre-LT treatment for HCC. Therefore, physicians should keep in mind that percutaneous locoregional therapy for HCC might cause such micro-dissemination with subsequent growth under immunosuppressive therapy. The value of adjuvant chemotherapy and choice of immunosuppressive agent such as Rapamycin needs to be further determined [22]. Although previous reports on pre-LT therapy basically tend to favor the treatment, the case documented above suggested some detrimental effect of percutaneous therapy for HCC.

With regard to the recurrence-free survival after LT, there was no significant difference between the patients with or without pre-LT therapy. Recently, Yao et al. [23] reported that after downstaging with pre-LT therapy in 61 patients the 1- and 4-year survival after LT was 96.2 and 92.1% respectively. There was no recurrence after LT. Overall, the recurrence-free survival after LT in patients after pre-LT treatment was as good as in patients with T2 HCC without therapy for HCC. The study cohort in the

current review were patients who received previous treatment for HCC, namely salvage liver transplantation. In comparison to down-staged patients, salvage LT patients should have a better survival since those patients have never demonstrated a condition beyond the Milan-Criteria. Therefore, the current results showing a good disease-free survival after LDLT is warranted.

Since the purpose of this study is to investigate the pre-LT treated lesions, we did not relate much information on untreated HCC. Investigation of untreated HCC and occult HCC were described by us recently [16]. The characteristics of the occult HCCs that were undetectable by imaging, included a minute (median size 6 mm), well-differentiated appearance (80%), with indistinct margins (85.3%) and without vascular invasion (94%). In the study, a multicentric occurrence of HCCs was demonstrated in cirrhotic livers with HCCs within the Milan criteria, although undetectable HCCs in cirrhotic livers may have no impact on recurrence after LT.

In conclusion, after pre-OLT therapy, 47% of the lesions still had viable HCC cells. However, pre-LT therapy for HCC in salvage LT had no effect on the outcome of LT. However, one case had peritoneal recurrence probably due to percutaneous locoregional therapy under immunosuppression.

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Original Article

Efficacy and limitation of bone marrow transplantation in the treatment of acute and subacute liver failure in rats

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Aim: Recent reports have shown that bone marrow cells (BMC) retain the potential to differentiate into hepatocytes. Thus, the BMC have been recognized as an attractive source for liver regenerative medicine. However, it has not been clarified whether BMC transplantation can be used to treat liver damage *in vivo*. In the present study, we explored whether BMC possess therapeutic potential to treat acute and/or subacute liver failure.

Methods: Fulminant hepatic failure (FHF) was induced by 70% hepatectomy with ligation of the right lobe pedicle (24% liver mass), followed by transplantation of BMC into the spleen. Dipeptidyl peptidase IV-positive (DPPIV$^+$) BMC were then transplanted into DPPIV-negative (DPPIV$^-$) recipients following hepatic irradiation (HIR) in which 70% of the liver was resected and the remnant liver irradiated.

Results: There was no benefit of BMC transplantation towards survival in the FHF model. DPPIV$^+$ hepatocytes appeared in the liver tissues of the DPPIV$^-$ HIR model rats, but DPPIV$^+$ hepatocytes replaced less than 13% of the recipient liver.

Conclusion: BMC transplantation may have limitations in the treatment of fulminant or acute liver failure because they do not have sufficient time to develop into functional hepatocytes. Preparative HIR may be beneficial in help to convert the transplanted BMC into host hepatocytes, and provide a survival benefit. Although, However, the precise mechanism warrants further studies.

Key words: bone marrow, fulminant liver failure, irradiation, liver regeneration

INTRODUCTION

WITH AN EVER-INCREASING shortage of donor organs for orthotopic liver transplantation, there is a significant need for alternative therapies for liver disease. Isolated hepatocyte transplantation has been successfully reported in experimental animals and in some clinical human cases.[1–3] However, the procedure of hepatocyte transplantation requires a great number of hepatocytes, and it is still uncertain as to whether or not the transplanted cells can actually engraft in the liver. Recent reports have shown that bone marrow cells (BMC) retain the potential to differentiate into a variety of non-hematopoietic cell lineages,[4–8] including hepatocytes. Thus, BMC have been recognized as an attractive cell source for liver regenerative medicine. For instance, Sakaida *et al.*[9] reported that BMC transplantation exhibited therapeutic potential by reducing liver fibrosis. This therapeutic potential against liver damage is considered to be due to differentiation to mature hepatocytes as well as improvement of intrahepatic micro-conditions. In addition, fusion between BMC and hepatocytes has been reported,[10,11] in which the fusion of host hepatocytes and donor BMC can give rise to mature hepatocytes without *trans*- or dedifferentiation. Such fusion is a new concept, but it is still unknown whether fusion is only a morphological phenomenon or whether it has a significant effect towards regeneration of the liver. On the other hand, previous reports have shown that *in vivo* cell fusion is an unlikely explanation for the "*trans*-differentiation" of bone marrow-derived cells into differentiated phenotypes.[12,13] In any case, investigation of BMC may contribute to the resolution of stem cells or progenitor cells which proliferate to mature hepatocyte, and may result in the promotion of cell transplantation study.

For clinical application, BMC transplantation has an advantage over other cell sources, for example,

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hepatocytes, embryonic stem cells and hepatic stem cells (oval cell or small hepatocytes), because: (i) the extraction technique of BMC has already been established by hematologists through bone marrow transplantation therapy; (ii) autologous transplantation can avoid rejection; and (iii) BMC may have the potential for self replication and aggressive proliferation.

In the present study, we explored whether BMC could have therapeutic potential in the two distinct, well-established, diseased liver models. One was the surgically induced fulminant liver failure model (FHF model) in which liver failure was caused by 68% hepatectomy followed by occlusion of the remnant liver lobes. The other was the hepatic irradiation model (HIR model), in which 68% hepatectomy was performed followed by irradiation of the remnant liver resulting in inhibition of liver regeneration.

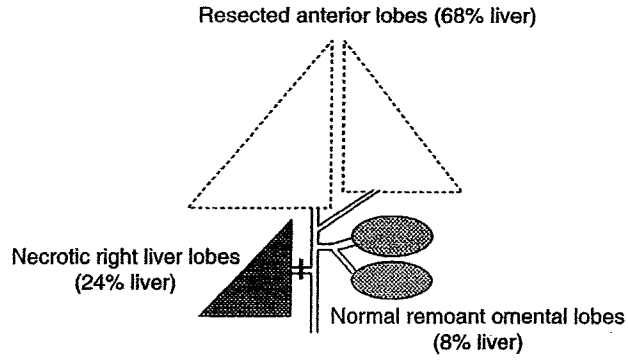

Figure 1 Schematic diagram depicting the technique used to induce the fulminant hepatic failure in this model. Two anterior lobes (68%; median lobe and left anterior lobe) were resected and the right lobes (24%) were rendered necrotic. Only the omental lobes (8%) were left intact.

METHODS

Animals

ADULT MALE SPRAGUE–DAWLEY (SD) rats and DPPIV-positive (DPPIV$^+$) 344 rats were purchased from Japan SLC (Shizuoka, Japan), while DPPIV-negative (DPPIV$^-$) 344 rats were purchased from Charles River Japan (Tokyo, Japan). All animal care and procedures were performed with the approval of the Nagasaki University Institutional Animal Care and Use Committee.

Surgical animal models

Induction of FHF model

Fulminant liver failure was induced as described previously by ourselves.[14,15] Briefly, the abdomen of male SD rats weighing 270–350 g (7–9 weeks old) was entered through a midline incision. The common pedicle to the right lobes of the liver (24% of the liver) was ligated, and the two anterior liver lobes (68% of the liver) were removed using the standard Higgins and Anderson technique.[16] The two omental liver lobes (8% of the liver) were left intact (Fig. 1). All surgical preparations and euthanasia were performed under general (diethyl-ether) anesthesia using sterile surgical technique. At the completion of the surgical procedure and every 12 h after the surgery, each FHF animal received a s.c. bolus of 10 mL of 5% dextrose in normal saline.

Induction of HIR model

The HIR model was generated as described previously.[17] Anesthesia was induced by i.p. injection (0.5 mL/kg pentobarbital). After aseptic preparation, 68% partial hepatectomy was performed through a midline incision. Immediately after partial hepatectomy, the animals were placed in a supine position on a surgical board. A jig with a 5 cm × 7 cm irradiation port was aligned to this platform. Two 1 cm × 2 cm lead shields, each 2 mm in thickness, were wedged under the liver to protect the stomach, kidney, spine and intestines, taking care not to compress the hepatic and aortic vessels. In addition, two lead shields were placed above the chest and the lower abdomen. A Toshiba EXS-300-5 was used (200 kVP, 10 mA, 0.5 mm aluminum, filtration, 43.5 cm SSD; dose rate, 3.2 Gy/min). The abdomen was then closed in two layers.

Bone marrow transplantation

Bone marrow cells were obtained from DPPIV$^+$ rats by flushing from the femurs with Dulbecco's modified Eagle medium (Sigma-Aldrich Japan, Tokyo, Japan) using a 21-G needle. The cells were filtered through a cell strainer (Falcon catalog no. 352350) and centrifuged at 1000 *g* for 5 min at 4°C, as previously described.[4] After washing with phosphate-buffered saline (PBS), pH 7.4, the cell pellet was suspended in 10 mL of lysis buffer (150 mM NH4Cl, 10 mM KHCO3, 0.1 mM ethylene diamine tetra acetate). After centrifugation, cells were washed twice in PBS, and the viability of BMC was confirmed to be more than 80% using Trypan blue dye exclusion.

A small left subcostal incision was made and the spleen was exposed. BMC (2×10^6 cells suspended in 0.5 mL of physiological saline) were injected under the fibrous capsule of the spleen. The numbers of trans-