Table 1. 肝臓移植のレシピエント適応基準

1. 余命と適応条件
   不治の末期状態にあり原則として従来の治療法では余命1年以内と予想されること．ただし，先天性肝・胆道疾患には必ずしも適応されない．
2. 年齢
   60歳未満が望ましい．
3. 適応疾患
   生体肝移植参照．
4. 絶対的除外条件
   ・他の主要臓器の進行した不可逆的障害
   ・全身・他臓器の活動性感染症（サイトメガロ感染症を含む）
   ・アルコールを含む薬物依存症
   ・HIV抗体陽性（ただし，現在は状況により判断される）
   ・肺内の右→左シャントによる強い低酸素脳症
5. 相対的条件については日本肝臓学会移植問題検討委員会において決定すること
6. 本人・家族の協力
   本人および家族の肝移植に対する十分な理解と協力が得られること．
7. 判定手続き
   内科系の関係学会認定専門医・指導医が外科系の関係学会認定専門医と協議の上，決定すること．

1993年移植関係合同委員会より抜粋．

難くない．また，今回提示したデータは，あくまでも脳死肝移植待機患者として登録された数であり，現状の提供者不足の中で「肝移植医療が成立する可能性がきわめて低いことより，登録にまで至らない（移植施設への受診することさえあきらめられた）患者さん」の数は，未知数である．提供数の増加が，待機時間の短縮に結びつくことで，肝移植医療が唯一の治療手段である患者さんたちの生命が救われるようになることを強く望みたい．

III 肝移植の適応基準[4]

脳死肝移植の適応基準の原則については，「不治の末期状態にあり原則として従来の治療方法では余命1年以内と予想されること」とされ，具体的には，Table 1に示される．脳死肝移植の適応になる代表的疾患は肝硬変であり，潜在的に多数のレシピエント候補を有する．特に本邦では，レシピエントの原疾患として約1/4を占める．この適応の現状については，登録基準における医学的緊急度評価にChild-Pugh分類が用いられる．末期肝硬変の肝機能評価において広く使用されているChild-Pugh分類であるが，その一方で評価項目の中で「腹水」，「肝性脳症」といった半定量的因子が含まれていることより客観性に欠けるという問題が指摘されている．

近年，アメリカでは，Child-Pugh分類による大まかな分類では，待機時間が優先権を決定する重要な因子となるため待機時間の死亡リスクを十分に反映できないという問題が生じたため，客観的で再現性の高いパラメータとしてMELD (model for end-stage liver disease) scoreを用いるようになった[8]．このことにより，肝移植患者の待機期間中の死亡症例は減少している[9]．本邦においても同様の問題点は以前より指摘されている．現時点においては，脳死肝移植適応評価においてMELD scoreを導入するか否かの結論は出てはいないが，今後本邦においてもこの点からの登録患者の適応評価が変更される可能性は十分にあり，できるだけ多くの患者さんが効率よく適正な順番で，脳死肝移植を受けられることを期待したい．

IV 肝移植実施施設

臓器移植法案・改正を受けて，現在脳死下での移植臓器提供者数は増加していることは先述した

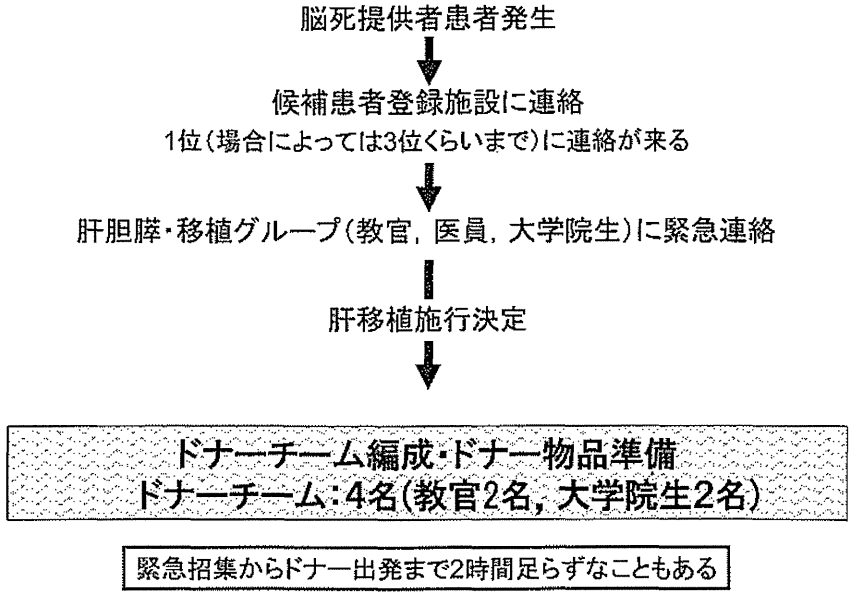

Figure 4. 臓器提供までの経過（大阪大学）.

とおりである．このことは，脳死肝移植医療が遅々として進行しなかった本邦では，何よりも喜ばしい事実である．また，脳死肝移植医療に対する関係各所の努力のたまものであることはいうまでもないし，進歩の1つである．

その一方で，最後にあげたいのは，移植施設側の問題である．現在，脳死肝移植認定施設は，全国に21施設（18歳未満限定の2施設を含む）ある（(社）日本臓器移植ネットワーク http//www.jotnw.or.jp/index.html）．これらの施設は，現在肝移植のみを施行しているわけではない．というのは，当科同様，ほぼ全施設において肝移植以外に肝胆膵外科診療に従事している．筆者らの施設（大阪大学消化器外科）においても，肝移植医療に従事するのは，上部消化管，下部消化管配属をのぞく，肝胆膵・移植グループに所属する消化器外科医（教官6名と医員5名）である．他の脳死肝移植認定施設同様，通常は，日本肝胆膵外科学会高度技能医修練施設（日本肝胆膵外科学会 http://www.jshbps.jp/）として，肝臓外科，胆道外科，膵臓外科，生体肝移植など，年間約200例の手術を施行している．その中で，脳死提供者が発生するとFigure 4に示す時間経過でドナー摘出チームを招集し，提供施設に摘出に向かう．出発までの準備時間が，数時間のことも少なくない．また，ほとんどの症例において脳死臓器提供者発生の連絡は午前2〜3時頃が多い．このため，人員の確保に難渋することもある．いずれにせよ，現在は本来研究に専念すべき大学院生の協力なしには，摘出チームの編成は困難である．また，Figure 5に示すように，約50kg以上の摘出手術関連物品を提供施設まで運搬する必要がある．その中には，保存液（UW 1L×6本）や保存液に入れる薬剤各種だけではなく，凍結生食（500ml×20パック）や冷却用氷（20kg）にくわえてスリッパ，マスク，帽子，手術用ガウン，手術用着，手袋，までが含まれる．このような状況が保険診療といえるのかどうか，これらの運搬に医師免許は必要なのかどうか，はなはだ疑問に感じているのは筆者らのみではないと思う．現在，教室では，これら器材・薬剤運搬も含めた緊急時の人員確保のために，2010年1月より，大阪市立大学と連携し，大阪大学附属病院・臨床登録医として摘出チームの一員として参加してもらっている．現在までに，2例の脳死肝移植実施症例において，摘出チームの人員不足においてご助力いただき，大変感謝している．今後，症例数の増加によっては，大阪府下の大阪医科大学，関西医科大学，近畿大学な

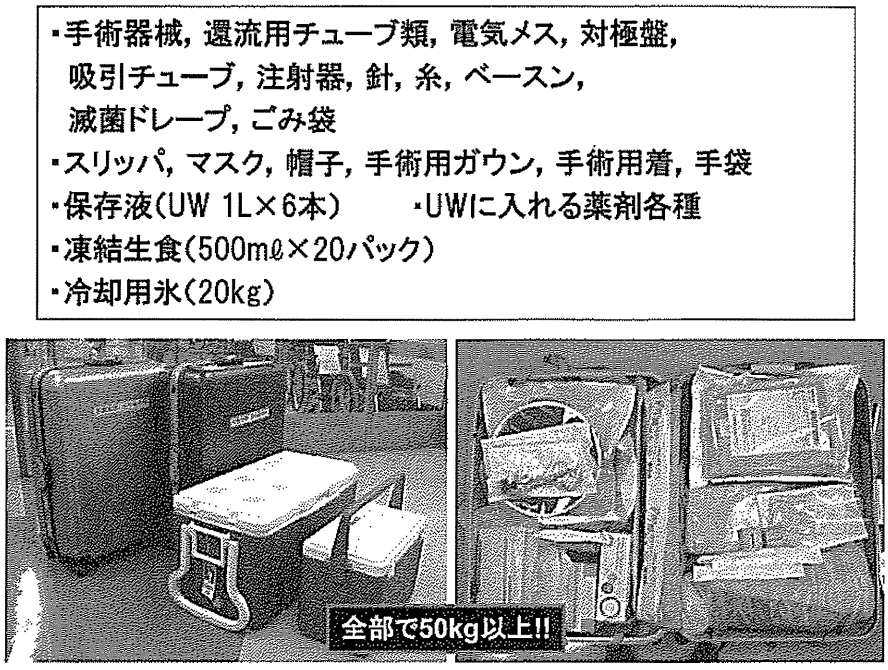

Figure 5. 肝提供者手術・準備物品．すべての準備物品は，大阪大学から提供施設へ医師4名で運ぶ．原則として，提供施設の物品は一切使用不可能．

ど他大学にもご協力をお願いし，脳死提供者摘出手術における，外科領域での診療連携を推進する必要性があるのではないかと考えている．

おわりに

近年，わが国の外科医療を取り巻く環境が大きく変化している．過剰労働や医療訴訟の増加などさまざまな要因により，外科志望者の減少が顕著となり，わが国の外科医数は1998年をピークに年々減少している．この外科医療崩壊の危機といっても過言ではない状況は脳死肝移植医療にも直結する．つまり，われわれがまさに直面している肝移植医療を志す外科医の激減である．このような状況への対策については，労働環境を改善することがその1つであることはいうまでもない[10]．特に脳死肝移植医療のように高度に複雑化された医療環境の中では，医療関係者それぞれの知識と専門性を生かした「チーム医療」が不可欠で，外科医の専門性を高め良好な就労環境で「肝移植医療」を展開することが最重要である．しかしながら，その現状にはほど遠く，提供者手術における50kgの器材・薬剤の運搬（Figure 5）はその象徴かもしれない．2007年4月日本外科学会は，第107回総会(会長：門田守人)において，今後の外科医療に対して，医療費，刑事司法，プロフェッショナリズムなどにくわえて，「医師に対する過重な負担を軽減するため，医師数の増加を図るとともに，コメディカルや医療事務等の充実により医師が本来業務に専念できるような体制を構築すべきである」との提言[1]を行った．脳死肝移植においても，移植外科医が減少し，その医療水準が維持できなくなるかもしれないという可能性がある今，移植医療のあるべき姿を考え，その構築に向けた取り組みを始めなければならない．改正臓器移植法案の施行が，表現は悪いかもしれないが，脳死肝移植を「お祭りから真の医療」に変える端緒になることを切に祈りたい．

本論文内容に関連する著者の利益相反
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ORIGINAL ARTICLE – HEPATOBILIARY TUMORS

Impact of Pegylated Interferon Therapy on Outcomes of Patients with Hepatitis C Virus-Related Hepatocellular Carcinoma After Curative Hepatic Resection

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ABSTRACT

Background. Several published reports investigating the effects of interferon (IFN) therapy on survival and tumor recurrence after curative resection of hepatocellular carcinoma (HCC) have been inconclusive. The aim of this study is to investigate the efficacy of pegylated-IFN (peg-IFN) therapy after curative hepatic resection for HCC in patients infected with hepatitis C virus (HCV).

Methods. Data from 175 patients who underwent curative hepatic resection for HCC associated with HCV were retrospectively collected and analyzed; 75 patients received peg-IFN therapy after surgery, whereas 100 patients did not receive IFN therapy. To overcome biases resulting from the different distribution of covariates in the two groups, a one-to-one match was created using propensity score analysis. After matching, patient outcomes were analyzed.

Results. After one-to-one matching, patients ($n = 38$) who received peg-IFN therapy after surgery and patients ($n = 38$) who did not receive IFN therapy had the same preoperative and operative characteristics. The 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.00135$). The 3- and 5-year overall survival rates were 100 and 91.7% and 76.6 and 50.6% in the peg-IFN group and non-IFN group, respectively. There was no significant difference in disease-free survival between the two matched groups ($P = 0.886$).

Conclusion. Peg-IFN therapy may be effective as an adjuvant chemopreventive agent after hepatic resection in patients with HCV-related HCC.

Hepatic resection is a well-accepted therapy for hepatocellular carcinoma (HCC), but many patients show cancer recurrence and the cumulative 5-year HCC recurrence rate exceeds 70%.[1–3] This high incidence of tumor recurrence after hepatic resection remains a major drawback. Some benefits of interferon (IFN) therapy on tumor recurrence and survival have been reported.[4–10] IFN suppresses replication of hepatitis C virus (HCV) and exerts a tumoricidal effect on a number of tumors, including HCC.[10,11] However, several randomized controlled trials (RCTs) have revealed inconclusive results regarding the effects of IFN on survival and tumor recurrence after curative resection or ablation of HCC, either because the effects were not statistically significant or because they were considered only with respect to defined subpopulations.[12–15]

Recently, combination therapy consisting of pegylated interferon (peg-IFN) plus ribavirin (RBV) has been developed, and the effect of this combination has been reported to be higher than that of conventional IFN therapy.[16,17] Peg-IFN has an extended serum half-life that provides viral suppression for 7 days, thus allowing weekly administration and enhanced clinical efficacy.[17] Most Japanese patients infected with HCV are infected with HCV genotype Ib and have high viral load. Moreover, treatment with conventional IFN is complicated by a low sustained viral response (SVR) rate of 20–30%.[18–20]

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However, peg-IFN plus RBV combination therapy has good tolerability in Japanese patients with HCV and resulted in an SVR rate of approximately 40–50%.[21–23] The impact of adjuvant immunotherapy with IFN after curative resection of HCC is debatable, and few studies have investigated the effects of peg-IFN plus RBV combination therapy on survival and recurrence after curative resection of HCC.

In the present study, we aim to investigate the impact of peg-IFN plus RBV combination therapy on survival and HCC recurrence after curative resection in patients infected with HCV.

PATIENTS AND METHODS

Patients and HCV Diagnosis

From June 2003 to June 2009, 370 HCC patients underwent hepatectomy as initial treatment at the Department of Gastroenterological Surgery, Hiroshima University Hospital, Japan. Of the 370 patients, 175 patients who were HCV RNA-positive/hepatitis B surface antigen-negative underwent curative hepatectomy. Of the 175 patients, 75 patients received IFN therapy after hepatectomy, and 100 patients did not receive any IFN therapy. Of the 75 patients who received IFN, 20 patients who received IFNs such as IFN-α or IFN-β were excluded. Of the 55 patients who received peg-IFN therapy, 43 patients who started peg-IFN within 9 months after curative resection were enrolled in this analysis. Twenty-four patients who had early recurrence of HCC within 9 months after surgery were excluded from the 100 patients who did not receive any IFN therapy, because these patients could lose the opportunity to receive IFN therapy for HCC recurrence if these patients were assigned to the peg-IFN therapy. Consequently, 119 patients were eventually enrolled in this study. Of these 119 patients, 43 received peg-IFN therapy within 9 months after hepatectomy, and 76 did not receive any IFN therapy.

Curative hepatectomy was defined as removal of all recognizable tumors. HCV RNA levels were measured by quantitative reverse-transcription polymerase chain reaction (RT-PCR; Amplicor, Roche Diagnostic Systems, CA, USA). HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region. HCV negativity was evaluated by quantitative RT-PCR. The lower limit of the assay was 5 kIU/ml (equivalent to 5,000 copies/ml) in the quantitative method and 50 IU/ml (equivalent to 50 copies/ml) in the qualitative method. SVR was defined as undetectable HCV RNA at 24 weeks after completion of IFN therapy. The study was approved by the concerned institutional review boards. Written informed consent was obtained from all patients.

Preoperative Diagnosis and Evaluation of HCC

Hepatocellular carcinoma was diagnosed on the basis of routine imaging modalities such as Doppler ultrasonography (US), computed tomography (CT) during hepatic angiography (CTHA) and CT during arterial portography (CTAP), and magnetic resonance imaging. Tumor stage, liver damage classification, and surgical procedures were defined according to the General Rules for Clinical and Pathologic Study of Primary Liver Cancer, fifth edition, by the Liver Cancer Study Group of Japan.[24]

Hepatectomy

The surgical procedure was determined according to tumor extent and hepatic reserve function. Liver function was assessed by liver damage classification, Child–Pugh classification, and indocyanine green retention rate at 15 min (ICGR 15).[25,26] If permitted by liver function, anatomic resection was performed.[27,28] In patients with insufficient hepatic reserve, limited resection was performed. We divided the liver parenchyma by using an ultrasonic dissector.[29] Postoperative complications were graded according to the method described by Clavien et al.[30]

Follow-Up

Follow-up evaluation after the surgery consisted of monthly blood chemistry tests and measurements of levels of tumor markers, including alpha-fetoprotein and des-gamma-carboxy prothrombin. Patients were examined by US every 3 months and by CT every 6 months. When recurrence was indicated by any of these examinations, patients were examined by CTAP and CTHA.

Patient Selection for IFN Therapy

Patients with HCV genotype 1b in the IFN group received peg-IFNα-2b (Pegintron; Schering-Plough, NJ, USA) at weekly dosage of 1.5 μg/kg subcutaneously for 48 weeks. Daily RBV (Rebetrol, Schering-Plough) was administered orally for 48 weeks, and the dosage was adjusted according to weight (600 mg for patients weighing $\leq$60 kg, 800 mg for those weighing 60–80 kg). Patients with HCV genotype 2 received IFN monotherapy for 24 weeks. Blood samples were obtained every 4 weeks and analyzed for HCV RNA levels. All patients were informed about IFN therapy after hepatectomy, and only consenting patients received IFN therapy. The eligibility criteria for IFN therapy were as follows: (1) detectable serum HCV RNA level, (2) Eastern Cooperative Oncology

Group (ECOG) performance score of 0 or 1, (3) platelet count $\geq$70,000/μl, (4) patients with no uncompensated cirrhosis (Child class C), and (5) hemoglobin concentration $\geq$10 g/dl. Peg-IFN therapy was commenced within 24 weeks of surgery or after the eligibility criteria were fulfilled.

Safety Assessments and Dose Modification of Peg-IFN Therapy

Adverse events were graded as mild, moderate, severe, or potentially life-threatening according to a modified World Health Organization grading system. The dose of peg-IFN was decreased by 50% and that of RBV was lowered to half in case of severe adverse events or when laboratory results revealed any of the following: hemoglobin concentration <10 g/dl in patients with no cardiac disease, decrease in hemoglobin concentration >2 g/dl in patients with cardiac disease, white blood cell count <3,000/mm^3, or platelet count <50,000/mm^3. Full dosage could be resumed on resolution of the adverse events. Treatment was permanently discontinued in case of life-threatening events or when laboratory results revealed hemoglobin concentration <7.5 g/dl after 4 weeks of dose reduction, white blood cell count <1,500/mm^3, or platelet count <30,000 mm^3.

Treatment for Recurrence

Patients with intrahepatic HCC recurrence were managed with ablative therapies such as radiofrequency ablation (RFA), percutaneous ethanol injection therapy, transarterial chemoembolization, or surgery including living-donor liver transplantation according to the tumor characteristics (number, size, and location of the tumors) and liver function.

Statistical Analyses

Categorical variables were compared using the chi-square test, and continuous variables were compared using the Mann–Whitney *U*-test. Overall survival and disease-free survival analyses were performed using Kaplan–Meier methods; comparisons between different groups were performed using the log-rank test. *P* value of less than 0.05 was considered significant. Calculations were performed using SPSS software (version 16; SPSS Inc., IL, USA).

Propensity analysis was performed using logistic regression to create a propensity score for the IFN and non-IFN therapy groups.[31,32] Variables entered in the propensity model were age, sex, HCV genotype, liver function test, tumor factors, and operative factors. The model was then used to provide a one-to-one match between the two groups by using the nearest-neighbor matching method.[33,34] Survival and disease-free survival analyses were performed in each matched subgroup to assess the impact of peg-IFN therapy on mortality after adjusting for the confounding factors.

RESULTS

Characteristics and Postoperative Course of the Entire Population

Differences in the characteristics of patients who received peg-IFN therapy after hepatic resection and those who did not receive IFN therapy after hepatic resection are presented in Table 1. Patients who received peg-IFN therapy were younger (65 vs. 71 years; $P = 0.0003$). Regarding tumor characteristics, there was no significant difference between the two groups. Operation times tended to be longer in patients who received peg-IFN therapy than in those who did not receive IFN therapy (260 vs. 242 min; $P = 0.05$). There were no hospital-related deaths in this study. Postoperative complications did not differ between the two groups. In the entire population, the 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.0024$) (Fig. 1a). However, there was no significant difference in disease-free survival between the two groups ($P = 0.795$) (Fig. 1b).

Results After Propensity Score Matching

Characteristics of the patients after propensity score analysis are presented in Table 1. Thirty-eight of the 43 patients who received peg-IFN therapy after hepatic resection and an equal number of the 76 patients who did not receive IFN therapy were matched after covariate adjustment. The study group of 76 patients was well matched; in particular, all covariates that significantly affected recurrence and postoperative liver failure in the entire study group were equally distributed between the two matched groups. Matched patients who received peg-IFN therapy after hepatic resection had similar total bilirubin and serum albumin levels and similar platelet counts to matched patients who did not receive IFN therapy. Similarly, the tumor characteristics, the surgical procedure, operation times, and blood loss during the operation in matched patients who received peg-IFN therapy were almost similar to those in patients who did not receive IFN therapy. There were no hospital-related deaths in the matched groups. Postoperative complications also did not differ between the two groups. The median follow-up period for patients who received peg-IFN and those who

TABLE 1 Baseline characteristics and operative data on patients who underwent hepatectomy: data are reported for whole study and for the matched study population after propensity score analysis

	Overall series		*P* value	Propensity-matched series		*P* value
	IFN (+) $n = 43$	IFN (−) $n = 76$		Peg-IFN (+) $n = 38$	IFN (−) $n = 38$	
Age (years)	65 (53–78)	71 (48–83)	0.0003	65.5 (53–75)	69 (51–80)	0.2
Sex (male/female)	27/16	47/29	0.918	23/15	25/13	0.634
Preoperative IFN	24 (55.8%)	29 (38.1%)	0.06	20 (52.6%)	14 (36.8%)	0.16
HCV genotype			0.876			0.6
1b	34	61		29	27	
2b	9	15		9	11	
Diabetes mellitus	11 (25.6%)	22 (28.9%)	0.856	11 (28.9%)	13 (34.2%)	0.621
ECOG PS			0.831			0.644
0	39	68		36	35	
1	4	8		2	3	
Platelet ($104/mm^3$)	10.3 (3.3–26.6)	10.3 (3.8–40.3)	0.381	9.75 (3.3–21.5)	11.2 (3.8–40.3)	0.454
T-Bil (mg/dl)	0.7 (0.3–1.4)	0.8 (0.3–1.7)	0.292	0.7 (0.4–1.4)	0.7 (0.3–1.7)	0.798
AST (IU/l)	42 (18–121)	48 (16–150)	0.152	43.5 (18–127)	41.5 (6–150)	0.567
ALT (IU/l)	38 (13–127)	41.5 (10–196)	0.987	40.5 (11–127)	37.5 (10–196)	0.226
Albumin (g/dl)	3.8 (2.8–5.2)	3.8 (2.5–4.9)	0.215	3.8 (2.8–5.2)	3.8 (2.5–4.5)	0.469
ICGR 15 (%)	17.9 (7.4–77.4)	18.7 (4.6–50.5)	0.734	17.65 (7.4–40.0)	17.55 (4.6–40.0)	0.561
AFP (ng/ml)	11.6 (0.5–3405)	27.6 (0.5–36572)	0.176	13.95 (0.5–3405)	22.9 (0.5–513)	0.635
Child–Pugh grade			0.665			0.556
A	41 (95.3%)	69 (90.8%)		37 (97.4%)	36 (94.7%)	
B	2 (4.7%)	7 (9.2%)		1 (2.6%)	2 (5.3%)	
Hepatic resection			0.322			0.373
Hr0	20 (46.5%)	49 (64.5%)		18 (47.4%)	23 (60.5%)	
HrS	13 (30.2%)	18 (23.7%)		12 (31.6%)	9 (23.7%)	
Hr1	3 (7.0%)	4 (5.3%)		2 (5.3%)	3 (7.9%)	
Hr2	7 (16.3%)	5 (6.6%)		6 (15.8%)	2 (5.3%)	
Hr3	0 (0%)	0 (0%)		0 (0%)	0 (0%)	
Operation time (min)	260 (128–623)	242 (90–580)	0.0514	257 (128–623)	247.5 (90–580)	0.18
Blood loss (ml)	200 (20–1900)	225 (10–960)	0.996	210 (20–1900)	210 (10–960)	0.803
Postoperative complications			0.933			0.798
IIIa	4	6		2	2	
IIIb	1	1		1	1	
IVa	1	1		1	0	
Stage			0.315			0.293
I	14 (32.6%)	19 (25.0%)		13 (34.2%)	9 (23.7%)	
II	18 (41.9%)	44 (57.9%)		15 (39.5%)	23 (60.5%)	
III	9 (20.9%)	12 (15.8%)		9 (23.7%)	6 (15.8%)	
IV-A	2 (4.7%)	1 (1.3%)		1 (2.6%)	0 (0.0%)	
Single tumor	28 (65.1%)	57 (75.0%)	0.252	25 (65.8%)	29 (76.3%)	0.312
Tumor size			0.712			0.589
≥3 cm	15 (34.9%)	24 (31.6%)		10 (26.3%)	8 (21.1%)	
<3 cm	28 (65.1%)	52 (68.4%)		28 (73.7%)	30 (78.9%)	
Vascular invasion	4 (9.3%)	3 (3.9%)	0.233	3 (7.9%)	0 (0.0%)	0.239

Continuous variables expressed as median (range)

Hepatic resection and stage were according to General Rules for the Clinical and pathological Study of Primary Liver Cancer, by Liver cancer Study Group of Japan, 5th edition, Kanehara Co., Ltd

Hr0: limited resection, HrS: segmentectomy, Hr1: sectionectomy, Hr2: hemihepatectomy, Hr3: more than hemihepatectomy

T-Bil total bilirubin, *PS* performance status, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *ICGR 15* indocyanine green retention rate at 15 min, *AFP* alpha-fetoprotein,

did not receive IFN therapy was 3.8 (1.2–6.9) and 3.5 (1.3–6.8) years, respectively. In the matched study groups, the 3- and 5-year overall survival rates of patients who received peg-IFN therapy after hepatic resection were significantly higher than those of patients who did not receive IFN therapy ($P = 0.00135$) (Fig. 1c). However, there was no significant difference in disease-free survival between the two matched groups ($P = 0.886$) (Fig. 1d).

In the matched 38 patients of the peg-IFN group, peg-IFN therapy was initiated at a median of 4.3 (0.9–9.6) months after hepatic resection. Thirty-one of 38 HCC patients began peg-IFN therapy within 6 months after hepatectomy. Seven patients required more than 6 months to commence peg-IFN therapy. Two patients required a longer time to recover platelet counts of more than 70,000/μl. Five patients required a longer time to decide to receive peg-IFN therapy. Sixteen (42.1%) of the matched 38 patients who received peg-IFN therapy after hepatectomy attained SVR. Among 16 patients who attained SVR, 10 patients received full-dose peg-IFN therapy without dose reduction, whereas 6 patients received a reduced dose of peg-IFN and/or RBV until completion of treatment. Nine patients discontinued peg-IFN therapy because of adverse events such as thrombocytopenia and neutropenia ($n = 2$), skin eruption ($n = 1$), depression ($n = 2$), and severe malaise ($n = 4$). Three patients discontinued peg-IFN therapy because of HCC recurrence. Adherence to peg-IFN therapy was 68.4% in this study. No life-threatening adverse events were observed, and none of the total 15 deaths in both sets of matched patients were related to the IFN treatment or to surgical procedures. The 3- and 5-year overall survival rates of patients ($n = 16$) who attained SVR after peg-IFN therapy were 100% and 100%, respectively; those of patients who did not attain SVR ($n = 22$) were 100 and 85.7%, respectively; and those of patients who did not receive IFN therapy were 76.6 and 50.6%, respectively. There was a statistically significant difference in overall survival among the three groups ($P = 0.005$) (Fig. 2a). However, there was no statistically significant difference in disease-free survival among the three groups ($P = 0.90$) (Fig. 2b).

Table 2 presents the patterns of cancer recurrence and the treatment details of the recurrences in both groups. Twenty-one (55.3%) of the patients who received peg-IFN therapy after hepatic resection and 17 (44.7%) of the patients who did not receive IFN therapy had HCC recurrences after hepatic resection. Regarding the pattern of recurrence, the proportion of patients who had multiple

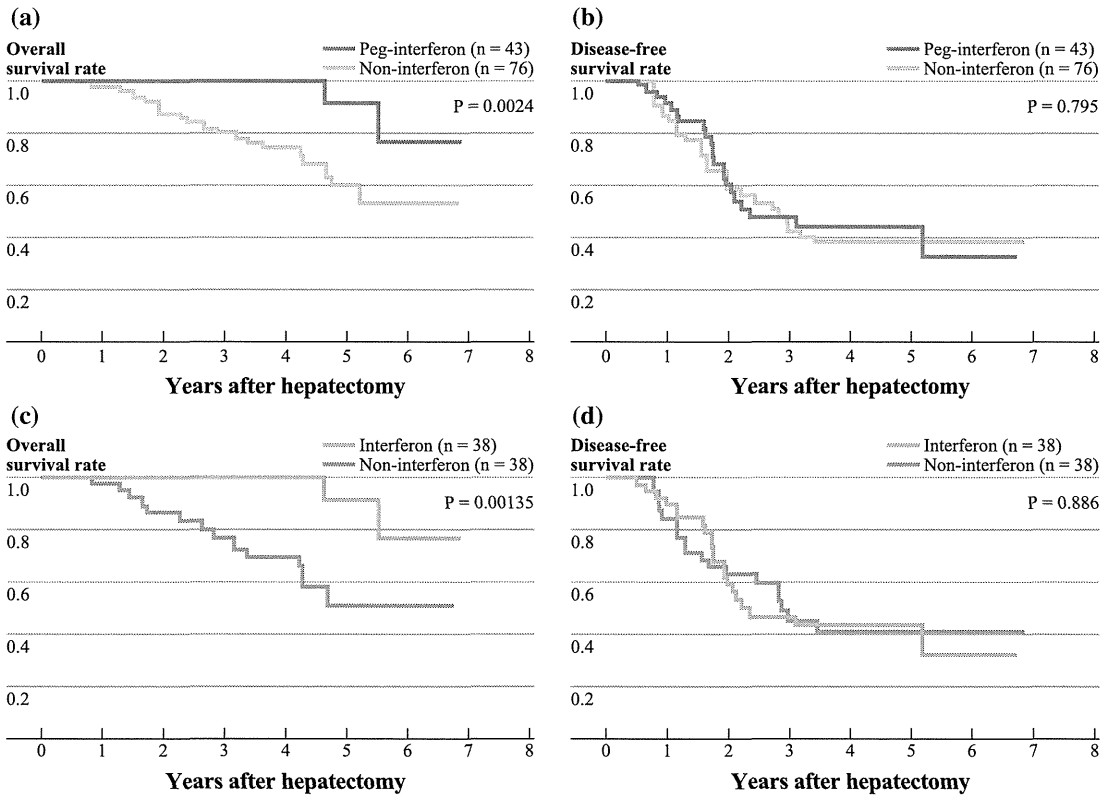

FIG. 1 Overall survival (**a**) and disease-free survival (**b**) of the entire study population of 175 patients with hepatitis C-related HCC with respect to IFN therapy after hepatic resection. Overall survival (**c**) and disease-free (**d**) survival of the matched study population of 76 patients with hepatitis C-related HCC with respect to IFN therapy after hepatic resection

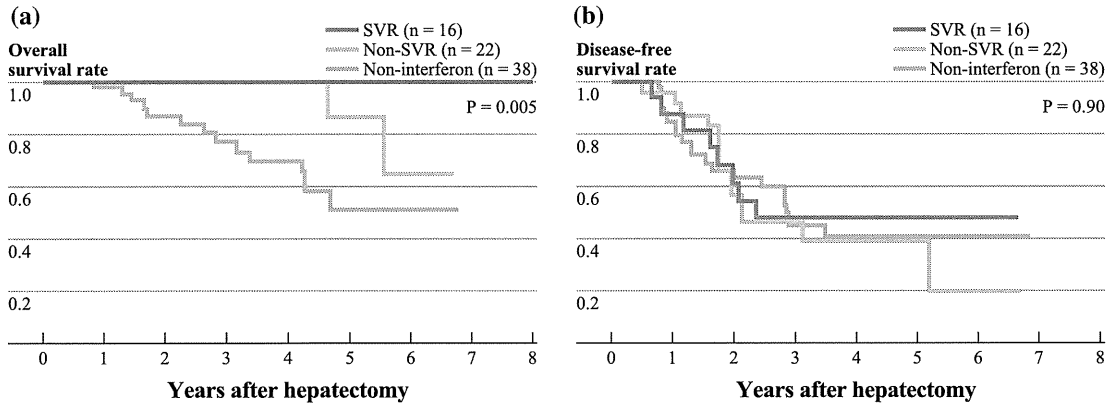

FIG. 2 Overall survival and disease-free survival of patients with hepatitis C-related HCC with respect to SVR after IFN therapy

intrahepatic recurrences (more than four nodules) was significantly lower in the peg-IFN group than in the non-IFN group ($P = 0.0047$). The proportion of patients in whom surgery or RFA was selected for treatment was significantly higher in the peg-IFN group than in the non-IFN group ($P = 0.0346$). Furthermore, regarding re-recurrence of HCC after treatment of the first-recurrent HCC, the 1-year disease-free survival rates of patients after treatment of the first-recurrent HCC was 48.5% in patients ($n = 21$) who received peg-IFN therapy and 12.5% in patients ($n = 17$) who did not receive IFN therapy. There was a statistically significant difference in disease-free survival between the two groups ($P = 0.0012$) (Fig. 3).

A comparison of results of the preoperative liver function test with those of postoperative 1-year liver function tests is presented in Table 3. In patients who received peg-IFN therapy, total bilirubin levels 1 year after surgery were significantly decreased compared with preoperative total bilirubin levels ($P = 0.018$), whereas in patients who did not receive IFN therapy, the total bilirubin level at 1 year after surgery was similar to the total bilirubin level before surgery ($P = 0.107$).

TABLE 2 Recurrence and treatments for recurrence after hepatic resection

	Peg-IFN (+) ($n = 38$)	IFN (−) ($n = 38$)	P value
HCC recurrence[a]: yes	21 (55.3%)	17 (44.7%)	0.359
Pattern of recurrence[b]			0.0047
Intrahepatic (single)	9 (42.9%)	8 (47.1%)	
Intrahepatic (2–3)	10 (47.6%)	1 (5.9%)	
Intrahepatic (multiple)	2 (9.5%)	8 (47.1%)	
Main modalities[b]			0.0346
Repeat hepatectomy	8 (38.1%)	2 (11.8%)	
RFA	8 (38.1%)	4 (23.5%)	
TACE	5 (23.8%)	11 (64.7%)	

peg-IFN pegylated interferon, *RFA* radiofrequency ablation, *TACE* transcatheter arterial chemoembolization

[a] Data expressed as number of patients (percentage of total patients)

[b] Data expressed as number of patients (percentage of patients who had a recurrence)

DISCUSSION

Our results revealed that peg-IFN therapy after hepatic resection improved the outcomes of HCV patients, although the interval of disease-free survival was not prolonged. Peg-IFN therapy after hepatectomy improved hepatic reserve function and suppressed multiple HCC recurrences (more than four nodules). Furthermore, re-recurrence after treatment of first-recurrent HCC after hepatic resection was significantly suppressed in the peg-IFN group compared with that in the non-IFN group. IFN has been reported to exert antitumor effects. IFN increases natural killer cell activity and exhibits antiangiogenic properties.[35,36] IFN has also been reported to be effective in eradicating HCV RNA

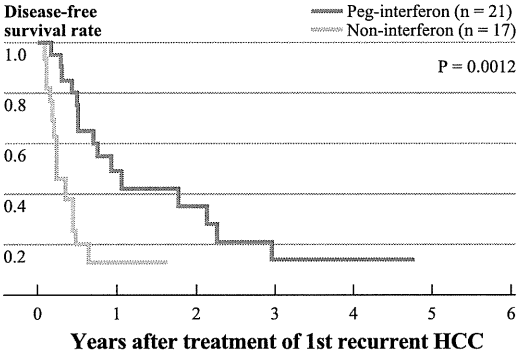

FIG. 3 Comparison of disease-free survival rate after treatment of first-recurrent HCC in patients who received peg-IFN therapy or in those who did not receive IFN therapy

TABLE 3 Comparison of preoperative liver function with 1-year liver function after hepatic resection

	Peg-IFN (+)		*P* value	IFN (−)		*P* value
	Preoperative	1 Year after surgery		Preoperative	1 Year after surgery	
T-Bil (mg/dl)	0.82 ± 0.29	0.71 ± 0.26	0.0189	0.81 ± 0.32	0.92 ± 0.35	0.107
AST (IU/l)	50.1 ± 24.1	45.8 ± 23.5	0.310	42.1 ± 18.9	56.1 ± 26.7	0.0110
ALT (IU/l)	51.3 ± 28.6	36.4 ± 22.8	0.00809	40.3 ± 24.3	49.7 ± 25.8	0.0918
Albumin (g/dl)	3.89 ± 0.80	3.99 ± 0.71	0.251	3.73 ± 0.45	3.75 ± 0.44	0.807

peg-IFN pegylated interferon, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase

from serum and hepatic tissue, thereby preventing deterioration of liver function in patients with HCV infection.[37] IFN prevents worsening of compensated cirrhosis.[18,37] Our results are compatible with those reported in those studies. In the peg-IFN group, most patients with HCC recurrence could undergo curative treatments such as repeat hepatectomy or RFA as a recurrence treatment, because the number of recurrent tumors was usually limited to three. IFN therapy appears to increase survival not only by improving residual liver function and increasing the possibility of radical treatment of recurrences but also by suppressing re-recurrence after the first recurrence of HCC.

The current study also revealed that the overall survival of patients with SVR was significantly better than that of patients without SVR. This result suggests that IFN prolongs the outcomes of patients with HCC after hepatic resection by causing remission of active hepatitis and eradication of HCV RNA in patients who attained SVR after hepatic resection.

In this study, to clarify the impact of peg-IFN therapy on outcomes of HCV-related HCC after hepatic resection, patients who received IFNs such as IFN-α or IFN-β were excluded. RCTs investigating adjuvant effects of IFN after resection or ablation of HCC were performed using IFN-α. Few studies have investigated the effects of peg-IFN plus RBV combination therapy on survival and recurrence after curative resection of HCC. Combination therapy with peg-IFN and RBV has recently been developed, and peg-IFN therapy has resulted in significantly higher SVR rates and better tolerability than treatment with IFN-α.[21,23] In our study, incidence of SVR after hepatic resection was 42.1%, which was higher than that in previous studies that reported an SVR rate of 0–10%.[12–14] The compliance of patients to peg-IFN therapy observed in the present study (68.4%) was higher than that reported elsewhere (approximately 40%).[14] This enhanced efficacy of the peg-IFN formulations might contribute to the prolonged survival of HCC patients after hepatic resection.

In this study, HCC patients who received peg-IFN therapy within 9 months after surgery were enrolled, and HCC patients who experienced recurrence of HCC within 9 months after hepatic resection were excluded from the non-IFN group, because these patients could lose the opportunity to receive IFN therapy for HCC recurrence on being assigned to the peg-IFN therapy group.

Before matching by using the propensity score, the clinical characteristics of the entire study population that can strongly influence outcomes differed significantly between the peg-IFN group and non-IFN group. The proportion of older patients was higher in the non-IFN group than in the peg-IFN group, whereas the proportion of patients who had longer operation times tended to be lower in the non-IFN group than in the peg-IFN group. To overcome bias due to the different distribution of the severity of liver function impairment between the two groups, a one-to-one match was created using propensity score analysis. After matching by propensity score, prognostic variables were appropriately handled, and there was no significant difference in prognostic factors between the two matched groups. This study had a limitation related to the small sample size after propensity score matching. To overcome this, further examination with larger sample sizes is necessary, and the potential efficacy of peg-IFN therapy must be validated in larger prospective RCTs.

CONCLUSIONS

Several previous RCTs investigating the effects of IFN on survival and tumor recurrence after hepatic resection were inconclusive. However, in the current study, peg-IFN therapy following hepatic resection improved the survival rates of hepatectomized patients with HCV-related HCC. The results of this study suggest that peg-IFN therapy is effective as an adjuvant chemopreventive agent after hepatic resection in patients with HCV-related HCC.

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CONFLICT OF INTEREST The authors have no commercial associations (e.g., consultancies, stock ownership, equity interest, patent/licensing arrangements) that might pose a conflict of interest related to the submitted manuscript.

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Hepatology Research

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Case Report

Eradication of hepatitis C virus genotype 1 after liver transplantation by interferon therapy before surgery: Report of three patients with analysis of interleukin-28 polymorphism, hepatitis C virus core region and interferon-sensitivity determining region

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The achievement of sustained viral response (SVR) with interferon (IFN) therapy before liver transplantation (LT) is difficult due to liver dysfunction, pancytopenia and frequent side-effects. Here, we report eradication of hepatitis C virus (HCV) genotype 1 after LT in three patients by IFN therapy before surgery. All three patients achieved virological response (VR), namely, fall in serum HCV RNA titer below the detection limit of real-time polymerase chain reaction (PCR) during IFN administration. However, HCV RNA rebound after cessation of treatment in all three patients; namely, they could not achieve SVR despite treatment with pegylated (PEG) IFN plus ribavirin. All three patients had wild-type amino acids (a.a.) at either aa70 or aa91 in the core region. Genotyping of IL-28 single nucleotide polymorphisms (rs8099917) showed TT genotype in two patients and TG genotype in one. All three patients developed multiple hepatocellular carcinomas during the clinical course, and requested living donor LT using liver grafts from their relatives. The patients were treated with IFN to immediately before LT, at which time they remained negative for HCV RNA in serum by real-time PCR. The three patients were followed-up for 14–15 months after LT, during which they remained negative for HCV RNA despite no further IFN therapy. In conclusion, it is possible to eradicate HCV after LT by inducing VR with continuous IFN therapy to before LT in spite of viral and host evidences reflecting low susceptibility to IFN treatment.

INTRODUCTION

RECENT RESULTS HAVE shown substantial improvement in the outcome of liver transplantation (LT). However, the outcome of LT for patients with hepatitis C virus (HCV)-related liver disease have been less satisfactory than those HCV negative individuals.[1–7] HCV recurrence is universal after LT with accelerated progression of liver fibrosis. Approximately 20–25% of HCV positive patients develop cirrhosis within 5–6 years after LT, and approximately 50% within 10 years.[5,8,9] Furthermore, the overall survival rate in these patients at 5 years after LT is poor, at approximately 60–70%.

In contrast, patients who have achieved HCV eradication before or after LT show longer survival.[2,3,10]

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However, eradication with interferon (IFN) therapy after LT is hampered by the use of immunosuppressive agents, anemia, frequent side-effects and the need to discontinue or reduce therapy. Even the results for antiviral therapy with pegylated (PEG) IFN plus ribavirin (RBV) in the transplant setting are poor, with sustained virological response (SVR) rates ranging 10–30% for genotype 1 HCV-infected patients.[11–17] Moreover, achieving SVR with IFN therapy before LT is also hampered by liver dysfunction, pancytopenia and frequent side-effects.[18,19] These findings highlight the need to establish effective protocols for the management of HCV recurrence in patients with LT.

Recent studies have shown that various host and virus factors are significant predictors of the efficacy of IFN treatment. With regard to viral factors, the number of amino acid (a.a.) substitutions in the IFN-sensitivity determining region (ISDR) (codon 2209–2248 or a.a. position 237–276 of the NS5A region) correlates with the SVR rate to IFN treatment in HCV genotype 1b patients.[20,21] Recent studies also reported that substitution of aa70 and/or aa91 in the HCV core region is an independent and significant predictor of virological response, such as SVR and non-virological response (NVR), to the combination therapy.[22–24] Furthermore, Okanoue *et al.*[25] also reported that the wild-type HCV core aa70 and two or more a.a. substitutions in the ISDR are useful markers for prediction of SVR.

On the other hand, human genetic factors such as single nucleotide polymorphisms (SNP) can be used to predict the effectiveness of IFN therapy. Polymorphisms in MxA,[26,27] IFN-α-receptor 1[28] and osteopontin[29] have also been reported to be associated with response to IFN therapy. We also identified MAPKAPK3 SNP[30] as a predictive factor for IFN monotherapy. Recent studies from three research groups reported independently that the response to PEG IFN plus ribavirin combination therapy in patients with HCV genotype 1b correlated with several SNP in the interleukin (IL)-28 locus.[31–35] However, there are no reports about correlation between IL-28 polymorphisms and viral eradiation after LT by treatment response to IFN therapy before LT.

Here, we analyzed a.a. substitutions in the HCV core region and ISDR by direct sequencing before living donor LT (LDLT) and analyzed IL-28 polymorphism in the recipients. The three patients continued IFN therapy until immediately before LT, at which time they remained negative for HCV RNA in serum by real-time polymerase chain reaction (PCR).

CASE REPORTS

Case 1

A 67-YEAR-OLD FEMALE with HCV-related liver cirrhosis later developed hepatocellular carcinoma (HCC). Platelet count was $11.9 \times 10^4/\mu L$, alanine aminotransferase (ALT) was 118 IU/L, genotype 1b, HCV RNA was 5.6 Log IU/ml, and Child–Pugh class was A. She had been treated with PEG IFN-α2b (60 μg) plus RBV (200 mg) for 24 months. Eight weeks after PEG IFN-α2b/RBV treatment, serum HCV RNA titer decreased below the detection limit (1.2 Log IU/mL). However, IFN therapy was stopped for treatment of HCC recurrence. This resulted in HCV RNA to become positive 4 weeks after cessation of the treatment. The presence of multiple HCC prevented surgical resection and ablation therapy. Instead, she received transcatheter chemoembolization (TACE). IFN therapy was restarted after TACE and serum HCV RNA titer became negative after 4 weeks of PEG IFN monotherapy. Her family requested LDLT donated by her daughter. LDLT was performed after obtaining informed consent. IFN therapy was continued for 4 months until 2 weeks before LT, and serum HCV RNA negativity by real-time PCR persisted until LT. The duration of VR was 4 months before LT. The patients had no a.a. substitutions in ISDR, and had mutant- and wild-type of a.a. at aa70 and aa91 in the core region, respectively. The patients had TG genotype of IL-28 SNP (rs8099917) (Table 1, Fig. 1a). She was followed up for 15 months after LDLT, and HCV RNA did not rebound during the follow-up period despite no further IFN therapy.

Case 2

A 60-year-old man with HCV-related liver cirrhosis later developed HCC. Platelet count was $11.8 \times 10^4/\mu L$, ALT 28 IU/L, genotype was 1b, HCV RNA was 4.6 Log IU/ml and Child–Pugh class was A. He underwent the combination therapy of PEG IFN-α2b (60 μg) plus RBV (200 mg, due to anemia) for 26 months. The HCV RNA titer decreased below the detection limit after 16 weeks of the combination treatment. However, PEG IFN-α2b/RBV was stopped for treatment of HCC recurrence. Tumor resection or ablation therapy was not possible due to the presence of multiple HCC and accordingly the patient underwent TACE. After TACE, HCV RNA became positive 4 weeks after cessation of PEG IFN-α2b/RBV treatment, necessitating resumption of the same treatment. Four weeks after the commencement of such therapy, HCV RNA became negative. The family requested LDLT using graft from his wife. IFN therapy

Table 1 Clinicopathological characteristic of the three patients

Case	Age/sex	Bodyweight (kg)	Genotype	HCV RNA (Log IU/ml)	Platelet count ($\times 10^4/\mu L$)	ALT (IU/L)	Child-Pugh	HCV mutations			IL-28B (rs8099917)	Dose of PEG IFN-α2b (μg)	Dose of RBV (mg)	Duration of		
								ISDR	HCV core aa70	HCV core aa91				IFN therapy (month)	VR before LT (month)	from last IFN to LT (wks)
1	67/F	57.1	1b	5.6	11.9	118	A	0	Mutant	Wild	TG	60	0	4	4	2
2	60/M	69.8	1b	4.6	11.8	28	A	0	Mutant	Wild	TT	60	800	3	3	2
3	54/M	63.8	1b	6.2	10.7	52	A	1	Wild	Mutant	TT	60	200	12	12	4

ALT, alanine aminotransferase; HCV, hepatitis C virus; IFN, interferon; IL, interleukin; ISDR, IFN-sensitivity determining region; LT, liver transplant; RBV, ribavirin; VR, virological response.

was applied for 3 months before LT, and serum HCV RNA became immediately negative as tested by real-time PCR and remained undetectable until LT. The duration of VR was 3 months before LT. The patients had no a.a. substitutions in ISDR, but had mutant-type and wild-type a.a. at aa70 and aa91 in the core region, respectively. Genotyping showed a TT genotype of IL-28 SNP (rs8099917) (Table 1, Fig. 1b). He was followed up for 14 months after LDLT, and HCV RNA did not rebound during the follow-up period despite no further IFN therapy.

Case 3

A 54-year-old man with HCV-related liver cirrhosis had received two courses of therapy for HCC. Platelet count was $10.7 \times 10^4/\mu L$, ALT 52 IU/L, genotype was 1b, HCV RNA was 6.2 Log IU/mL, and Child–Pugh class was A. Though he achieved VR, SVR could not be attained after 48-week PEG IFN plus RBV therapy. IFN therapy was subsequently withheld for treatment of HCC recurrence. Surgery with curative intent was deemed not possible due to the presence of multiple HCC, and instead treated by TACE. After TACE, retreatment with PEG IFN-α2b (60 μg) plus RBV (200 mg) was restarted. At the wishes of his family, he underwent LDLT using graft from his son. IFN therapy was continued just before LDLT. The duration of VR before LT was 12 months. The patient had one a.a. substitution in ISDR, and wild- and mutant-type a.a. at aa70 and aa91 in the core region, respectively. Genotyping showed TT genotype of IL-28 SNP (rs8099917) (Table 1, Fig. 1c). He was followed up for 15 months after LDLT, and HCV RNA did not rebound during the follow-up period despite no further IFN therapy.

DISCUSSION

THE OUTCOME OF LT in patients with HCV-related liver disease is poorer than that of patients with other etiology. The most common reasons for the poor performance are HCV recurrence and fibrosing cholestatic hepatitis. These findings highlight the need to establish effective protocols for the management of HCV recurrence after LDLT. The management of patients with HCV-related liver disease who undergo LT includes treatment of both pre- and post-LT HCV. Treatment of HCV before LT is limited by poor tolerance.[18] The side-effects of IFN therapy include bacterial infection, and thus such therapy should be adequately monitored. Review of previous reports on IFN therapy before LT[18,19,36–38] shows virological response at the time of LT in 61 patients treated with IFN, of whom 42 (68.8%)

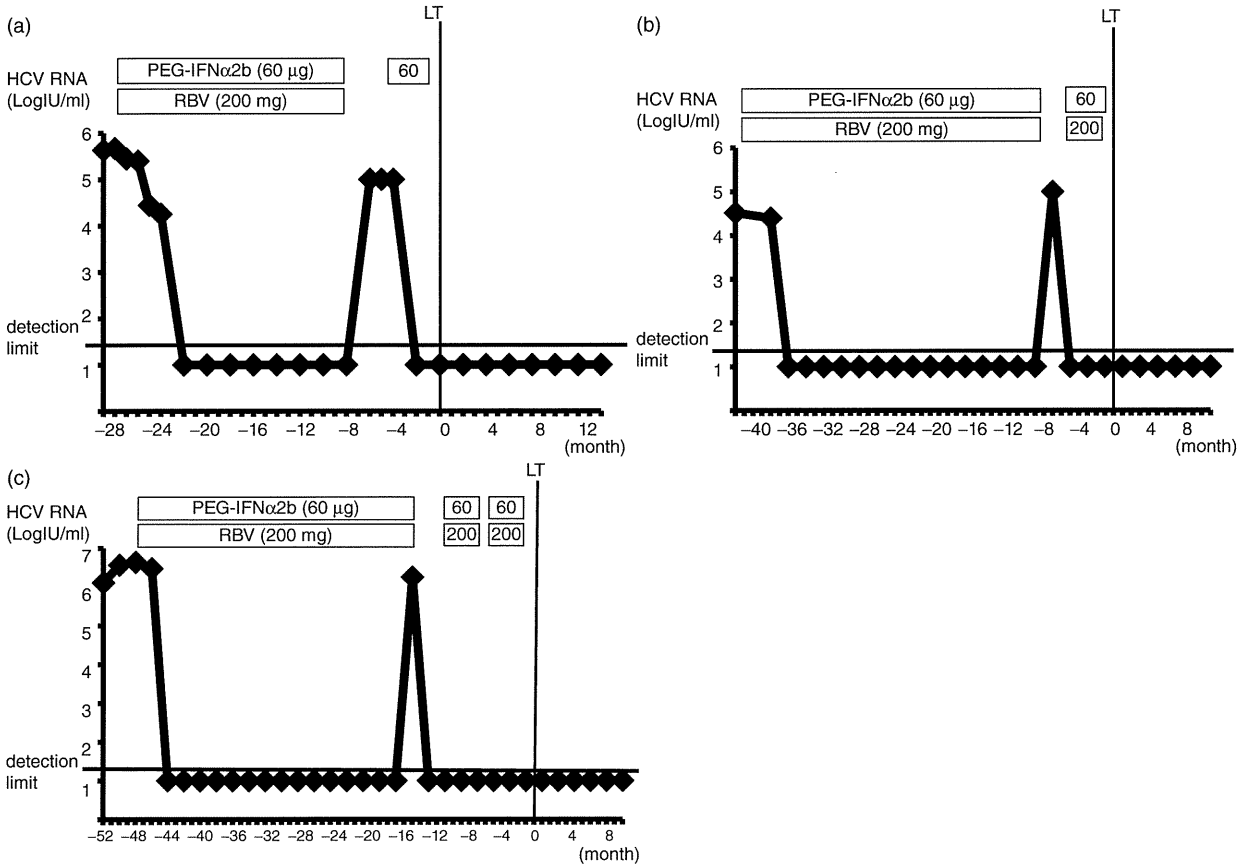

Figure 1 (a) Case 1. Changes in hepatitis C virus (HCV) RNA titer in response to treatment with the combination of pegylated interferon-α2b (PEG IFN) plus ribavirin (RBV). (b) Case 2. Changes in HCV RNA titer in response to treatment with the combination of PEG IFN plus RBV. (c) Case 3. Changes in HCV RNA titer in response to treatment with the combination of PEG IFN plus RBV.

achieved HCV eradication after LT. However, because of the lack of details about previous IFN therapy, these patients might have achieved SVR at stopping IFN therapy before LT. In support of this, another study that examined the presence of HCV RNA in liver explants showed no HCV RNA in 11 of 17 (64.7%) patients,[38] while six had detectable HCV RNA. Eradication of HCV after LT was reported in two of these six patients while re-infection after LT occurred in the other four. However, there was no detail information about VR duration and viral factors in these patients.

In the present study, although our three patients achieved virological response during IFN therapy, they did not achieve SVR due to HCV relapse after the withdrawal of IFN therapy. The VR in these patients continued until the time of LDLT, and HCV was eradiated after LDLT in all three. It was considered that HCV RNA titer was zero or markedly under the detection limit in serum, though HCV RNA persisted in the liver tissue. These findings suggest that HCV might have been eradicated from whole body at the time of LT. On the other hand, previous reports suggested that HCV replication also occurs in other organs such as the lymph node, bone marrow and brain.[38–40] Although HCV RNA titer was zero or markedly below the detection limit in serum, viral relapse could occur after LT if the above organs are positive for HCV RNA. Because HCV RNA was negative in these organs, it is highly likely that HCV was eradicated from the whole body at the time of LT in the three patients.

Recent studies have identified various host and virus factors as significant predictors of the response to IFN

treatment. With regard to the viral factors, the number of a.a. substitutions in the ISDR correlates with the SVR rate to IFN treatment in patients infected with HCV genotype 1b.[20,21] In a series of studies, Akuta *et al.*[23] reported that substitution of aa70 and/or aa91 in the HCV core region is an independent and significant predictor of virological response, including SVR and NVR, to the combination therapy. Other studies also reported the association between several SNP in the IL-28 locus and the effect of PEG IFN plus ribavirin combination therapy in patients with HCV genotype 1b.[31–34] In our patients, the numbers of a.a. substitutions in ISDR were 0–1 together with mutant-type a.a. at either aa70 or aa91 in the core region, which showed low susceptibility to IFN treatment. Furthermore, genotyping of IL-28 SNP (rs8099917) was TT genotype in two patients and GG genotype in one patient.

It is possible that these viral factors are not linked to the achievement of SVR with PEG IFN/RBV treatment for HCV infection before LT. In this regard, one previous study reported a viral response rate of 60–70% in patients with chronic hepatitis at the end of PEG IFN plus RBV treatment.[41–43] In other words, 60–70% of patients could attain viral response in spite of viral and host factors. Thus, these results suggest that it is better to treat the patients with PEG IFN plus RBV before LT, if possible. Should VR be achieved, continuation of IFN therapy to immediately before LT could result in HCV eradication after LT.

Of course, we do not recommend IFN therapy for all patients. Smallwood *et al.*[44] reported that IFN therapy before LT for HCV patients was associated with poor outcome after LT. However, among LT candidates, some patients may show viral response but not SVR. In addition, some patients can continue the IFN therapy even after the appearance of side-effects that are within the allowable limits. Further clinical trials of larger population samples are necessary to confirm the present findings.

In conclusion, the three cases presented here suggest that patients who show viral response to IFN therapy before LT can escape recurrence of HCV after LT due to the continuation of IFN therapy to just before LT.

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IL28B polymorphism may guide pegylated interferon plus ribavirin therapy even after curative treatment for hepatitis C virus-related hepatocellular carcinoma

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SUMMARY. The present study was designed to determine the predictive factors for the viral response to pegylated interferon-alpha plus ribavirin combination therapy (PEGIFN/RBV) administered after curative treatment for hepatitis C virus (HCV)-related hepatocellular carcinoma (HCC). The study group was 78 patients treated between January 2005 and January 2009. The sustained viral response (SVR) rate was 25.8% (15/58) in patients infected with HCV-genotype 1 and 55.0% (11/20) in those with genotype 2. Among the 78 patients, 32 (41.0%) could not complete the treatment protocol, and this was because of HCC recurrence in 17 (53%) of them. Multivariate analysis identified partial early viral response (pEVR) as the only independent determinant of SVR [odds ratio (OR) 14.73, $P = 0.013$] for patients with genotype 1. Multivariate analysis identified male gender (OR 8.72, $P = 0.001$) and interleukin-28B (IL-28B) genotype (rs8099917) TT (OR 7.93, $P = 0.007$) as independent predictors of pEVR. Multivariate analysis also identified IL-28B genotype GG+TG (OR 14.1, $P = 0.021$) and α-fetoprotein >30 (OR 5.4, $P = 0.031$) as independent predictors of null response. Patients with SVR showed a better survival rate than those without SVR ($P = 0.034$). The second HCC recurrence rate tended to be lower in patients with SVR than in those without SVR ($P = 0.054$). With regard to the prognosis of patients with SVR, it is desirable to achieve SVR with interferon therapy even when administered after HCC treatment. IL-28B genotype is a potentially useful marker for the response to PEGIFN/RBV therapy administered after curative treatment of HCV-related HCC.

Keywords: curative treatment, hepatitis C virus, hepatocellular carcinoma, interleukin-28B, pegylated interferon-alpha plus ribavirin combination therapy.

INTRODUCTION

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide. Chronic infection with hepatitis C virus (HCV) has been associated with hepatocarcinogenesis [1–3]. Recent advances in imaging and treatment modalities have brought about some improvement in the prognosis of patients with HCV-related HCC, but the overall outcome remains unsatisfactory; the 5-year survival rate is only 50–70%, even after curative treatment such as hepatic resection or local ablation [4]. The reasons for this unfavourable prognosis are considered to include high intrahepatic tumour recurrence rates and sustained hepatic damage, both resulting from HCV infection [5]. Even after curative hepatic resection for HCV-related HCC, the rate of intrahepatic tumour recurrence within 1 year is 20–40%, rising to about 80% by 5 years [4,6,7].

Intrahepatic recurrence of HCC may result from intrahepatic metastasis originating from the primary HCC or from ongoing multicentric carcinogenesis related to chronic HCV infection. The background HCV-related hepatic damage may

Abbreviations: AFP, α-fetoprotein; cEVR, complete early viral response; HCV, hepatitis C virus; IFN, interferon; IL-28B, interleukin-28B; NR, null response; PEGIFN, pegylated interferon; PEGIFN/RBV, pegylated interferon-alpha plus ribavirin combination therapy; pEVR, partial early viral response; RBV, ribavirin; SNP, single-nucleotide polymorphism; SVR, sustained viral response; TR, transient viral response.

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also compromise hepatic functional reserve and worsen clinical outcome. Thus, the prevention of HCC recurrence as well as preservation of liver function constitutes high priorities for the improvement of prognosis of patients with HCV-related HCC.

Interferon (IFN) therapy for patients with HCV infection is effective in reducing serum alanine transaminase (ALT) activity and in eradicating HCV [8,9] and thus IFN could be of value in minimizing hepatic necrosis, inflammation and fibrosis, as well as reducing the incidence of HCC. Several recent studies have reported that IFN therapy, applied even after curative treatment for HCV-related HCC, could prevent HCC recurrence and improve survival [10–21].

The recent introduction of pegylated interferon-alpha plus ribavirin combination therapy (PEGIFN/RBV) has improved the treatment efficacy [22,23]. Recent studies have highlighted the relationship between various single-nucleotide polymorphisms (SNPs) in the IL-28 locus and the effect of PEGIFN/RBV in patients infected with HCV [24–29]. Further, the results of few recent studies suggest that PEGIFN/RBV could prevent HCC recurrence and improve survival even when used after curative treatment of HCV-related HCC [30,31]. To our knowledge, however, there are no studies on the factors that could predict a sustained viral response (SVR) to PEGIFN/RBV after treatment of HCC (e.g. IL-28B as a host factor). The present study was designed to determine the predictive factors of viral response to PEGIFN/RBV in patients with HCV treated for HCC.

MATERIAL AND METHODS

Patients

The study subjects were 78 patients treated with PEGIFN/RBV after curative intent treatment (hepatic resection or radiofrequency ablation) for HCV-related HCC between January 2005 and January 2009 in this retrospective cohort study. Tumour staging was defined based on the Liver Cancer Study Group of Japan/Tumour-Node-Metastasis staging system of the Liver Cancer Study Group of Japan (LCSGJ): stage I [fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion, $n = 28$ (36%)], stage II [two of the three intrahepatic conditions, $n = 27$ (35%)], stage III [one of the three intrahepatic conditions, $n = 23$ (29%)], stage IVa (none of the three intrahepatic conditions, with no distant metastases or any intrahepatic conditions with lymph node metastases) and stage IVb (any intrahepatic condition with distant metastases) [stage IV, $n = 0$ (0%)] [32]. The median duration was 7 months (range, 1–60) from curative intent treatment for HCC to the start of PEGIFN/RBV therapy.

Antiviral treatment protocol

Each patient received 1.5 μg/kg body weight (BW) pegylated interferon-alpha (PEGIFN) (Peg-Intron; Schering-Plough, Segrate, Italy) subcutaneously (s.c.) once weekly, together with ribavirin (RBV) (Rebetol; Schering-Plough). The RBV dose was adjusted according to BW to 600 mg for patients <60 kg BW, 800 mg for >60 but ≤80 kg BW and 1000 mg for >80 kg BW, based on the drug information for RBV supplied by the manufacturer. The above durations and dosages are those approved by the Japanese Ministry of Health, Labour and Welfare.

The daily dose of RBV was reduced by 200 mg when haemoglobin (Hb) fell below 10 g/dL, acute fall in Hb concentration followed by stabilization at more than 3 g/dL from baseline, or appearance of clinical symptoms of anaemia (e.g. fatigue, pallor, palpitation, dyspnoea on efforts and fatigue) associated with a decrease in Hb of >2 g/dL from baseline. Once the RBV dose was reduced, it was maintained at that level throughout the rest of study. The protocol also included withdrawal of RBV when Hb fell below 8.5 g/dL or when patients manifested more severe anaemia including orthostatic hypotension. After the end of the treatment, the patients were followed up for 24 more weeks without treatment. The treatment term was 48 weeks for patients infected with HCV genotype 1 and 24 weeks for those with genotype 2.

The study was conducted in accordance with the Declaration of Helsinki and was approved by the local Ethics Committees of all participating centres. Written informed consent was obtained from each patient. At each visit, information on possible side effects was obtained by questioning the patients in a structured manner about specific, commonly observed and expected side effects of the study medication, such as flu-like symptoms, fatigue, nausea, vomiting, diarrhoea, dizziness, depression and hair loss.

Single-nucleotide polymorphism genotyping and quality control

Because the two reported significant IL-28 SNPs (rs8099917 and rs12979860) are in strong linkage disequilibrium, we analysed only rs8099917 in this study. Some samples obtained from patients with HCV were determined using the Illumina HumanHap610-Quad Genotyping BeadChip, whereas the remaining samples were genotyped using the Invader assay, as described previously [33,34].

Analysis of nucleotide sequence of the core and NS5A region

The amino acid (aa) substitutions at aa 70 and aa 91 of the HCV core region and mutation at the interferon sensitivity-determining region (ISDR) in the nonstructural 5A (NS5A) region of HCV were analysed by the direct sequencing method as described previously by our group [35–37].

Assessment of viral response

Serum HCV RNA was determined at baseline, after 4, 8, 12, 16 and 20 weeks of treatment, at the end of treatment and