pretreatment predictive factors of response, time to progression (TTP), and survival of HCC patients treated with the combination therapy remain unclear. At present, some patients with nonresectable HCC are treated with TACE. However, some patients are not suitable candidates for TACE because of PVTT or poor response to TACE. Because of the poor prognosis of patients with nonresectable HCC who are not treatable by TACE, effective treatment is needed. There is little information about assessment of patients with advanced HCC (e.g., nonresectable HCC with PVTT in the second branch or nonresectable HCC without PVTT but with poor response to TACE) treated with combination therapy of intraarterial 5-FU and IFN. In the present retrospective cohort study, we assessed the efficacy of intraarterial 5-FU with IFN for various types of nonresectable advanced HCC and investigated the pretreatment predictive factors of early response, TTP, and survival in response to the combination therapy.

Materials and methods

Patients

From June 2003 to December 2006, 265 consecutive patients with unresectable HCC were admitted to our hospital. Of the 265 patients with advanced HCC, 94 were treated with TACE, 34 patients received systemic chemotherapy, and 13 patients received best supportive care. The remaining 124 patients were selected as suitable candidates for intraarterial 5-FU and IFN combination therapy. Forty-one patients refused the therapy. Thus, 83 patients with advanced HCC were treated with intraarterial 5-FU and IFN. Of these 83 patients, 24 with distant metastases and four with hepatic venous invasion were excluded from this study, so we assessed 55 patients without distant metastases or hepatic venous invasion in this retrospective cohort study. Of the 55 patients, 30 had been treated with TACE before enrollment. Table 1 lists the baseline characteristics of the 55 patients. PVTT grade, based on the location of the tumor thrombus, was determined according to the criteria of the Liver Cancer Study Group of Japan (LCSGJ).[18] PVTT grading was as follows: Vp 0, no PVTT; Vp 1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp 2, tumor thrombus in a second branch of the portal vein; Vp 3, tumor thrombus in the first branch of the portal vein; and Vp 4, tumor thrombus in the trunk of the portal vein. Tumor staging was defined based on the TNM staging system of the LCSGJ:[18] stage I (fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion), stage II (fulfilling two of the three intrahepatic conditions), stage III (fulfilling one of the three intrahepatic conditions), stage IVA (fulfilling none of the three intrahepatic conditions with no distant metastases or any intrahepatic conditions with lymph node metastases), and stage IVB (any intrahepatic conditions with distant metastases).

Eligibility

This was a retrospective cohort study to investigate pretreatment predictive factors of TTP, survival, and

Table 1. Clinical profile of the 55 HCC patients

Age (years)[a]	67 (38–79)
Sex (M/F)	44/11
Etiology: HBV/HCV/other	15/36/4
Total bilirubin (mg/dl)	1.1 (0.4–6.4)
Platelet count ($\times 10^4$ mg/dl)	13.0 (5.1–54.5)
Albumin (mg/dl)	3.5 (2.4–4.8)
Child Pugh stage (A/B/C)	43/10/2
PS (0/1)	45/10
Intrahepatic tumor volume (≤50%/>50%)	38/17
Tumor stage (III/IVA)	20/35
Vp[a] (0/2/3/4)	20/6/15/14
AFP (ng/ml)	934 (14.3–525 900)
AFP-L3 (%)	47.3 (<0.5–87.6)
DCP (mAU/ml)	3729 (10–722 140)
Previous treatment (performed/not performed)	30/25

Data are expressed as median with range values in parentheses, or number of patients HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HBV, hepatitis B virus; PS, Eastern Cooperative Oncology Group performance status; AFP, α-fetoprotein; AFP-L3, lens culinaris agglutinin-reactive fraction of α-fetoprotein; DCP, des-γ-carboxy prothrombin; PVTT, portal vein tumor thrombosis

[a] PVTT grade: Vp 0, no PVTT; Vp 1, tumor thrombus in a third or more of the peripheral branches of the portal vein; Vp 2, tumor thrombus in a second branch of the portal vein; Vp 3, tumor thrombus in the first branch of the portal vein; Vp 4, tumor thrombus in the trunk of the portal vein

response to intraarterial 5-FU/IFN combination therapy. Eligibility criteria were as follows: age, 18–80 years; leukocyte count, >2000/μl; neutrophil count, >1200/μl; hemoglobin, >8 g/dl; platelet count, >50 000/μl; unresectable or not suitable for local ablation therapy, including RFA or PEI; with PVTT or TACE was ineffective; without hepatic venous invasion; without distant metastases; and Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0–1.[19] There was no eligibility criterion regarding hepatic reserve function, including serum total bilirubin levels. All patients gave written informed consent to this study, which was approved by the Institutional Review Board of Hiroshima University.

Treatment protocol

Patients received repeated arterial infusions of anticancer agents via the injection port. One course of chemotherapy lasted 4 weeks. 5-FU (500 mg body weight/day, Kyowa Hakko, Tokyo, Japan) was administered over 5 h with a mechanical infusion pump on days 1 to 5 of the first and second weeks (5 g in one course). Recombinant IFN α-2b (Intron A, Schering-Plough Pharmaceuticals, Osaka, Japan); 3×10^6 U (3 MU), or natural IFN α (OIF, Otsuka Pharmaceuticals, Tokyo, Japan); 5×10^6 U (5 MU) was administered intramuscularly on days 1, 3, and 5 of each week (total dose, 36 and 60 MU, respectively). In principle, treatment was repeated several times unless PS changed to 3 or 4 during the treatment. A 2- to 4-week rest period of no treatment was allowed after each treatment course. As for the two types of IFN, we previously reported similar effects of recombinant IFN α-2b and natural IFN α when combined with intraarterial 5-FU for the treatment of advanced HCC.[20]

Implantation of the arterial catheter

A catheter was inserted through the right femoral artery by the Seldinger method. After localization of the HCC, a 3-French heparin-coated catheter was inserted and its tip advanced to the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port, which was implanted in a subcutaneous pocket created in the right lower abdominal quadrant. The gastroduodenal artery and right gastric artery were occluded with steel coils to prevent gastroduodenal injury by the chemotherapeutic agents.

Evaluation

The early response to the combination therapy was assessed with contrast-enhanced CT after two courses of the combination therapy. The response was defined according to the criteria of the Response Evaluation Criteria in Solid Tumors (RECIST).[21] A complete response (CR) was defined as the complete disappearance of all target lesions. A partial response (PR) was defined as a decrease of at least 30% in the sum of the longest diameter of the target lesions with the baseline sum of the longest diameter of the target lesions as the reference. Progressive disease (PD) was defined as an increase of at least 20% in the sum of the longest diameter of target lesions. Stable disease (SD) was defined as meeting neither the PR nor the PD criteria. The duration of the response was measured from the date of the start of treatment to the date of documented progression. Adverse reactions were assessed with the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 3.0)[22] every week during the treatment.

Additional therapy

After two courses of the combination therapy, we assessed the response to therapy in all patients. According to the response, we provided various additional therapies such as RFA, TACE, or radiotherapy (RT) to patients treated with the combination therapy. These additional therapies were considered for patients with PS of 0–1 and a Child-Pugh stage of A or B. Patients assessed with PR continued to receive the combination therapy repeatedly. Then, when downstaging of advanced HCC was achieved (single tumor ≤50 mm in diameter or 1–3 tumors ≤30 mm in diameter) by the repeated combination therapy, RFA was considered. For patients assessed with SD or PD, in addition to the combination therapy, TACE with cisplatin–lipiodol suspension was performed. The catheter tip was advanced superselectively into the feeding artery so that sufficient anticancer agent was delivered. Among the patients assessed with SD or PD, RT was performed for PVTT if present. For patients assessed with CR, the clinical course was observed without adjuvant chemotherapy or additional therapy.

Statistical analysis

Statistical analysis was performed on 1 April 2007. Differences between groups were examined for statistical significance using the Mann-Whitney *U* test, logistic regression test, or χ-squared test as appropriate. Cumulative survival rate and TTP were calculated from the initial date of the combination therapy and assessed by the Kaplan-Meier life-table method, and differences were evaluated by the log rank test. Univariate and multivariate analyses of predictors for early response to the combination therapy were assessed by logistic

regression test. Univariate analysis of predictors of TTP and survival of patients with HCC who received the combination therapy was assessed by the Kaplan-Meier life-table method, and differences were evaluated by the log rank test. Multivariate analysis of predictors of TTP and survival was assessed by Cox proportional hazard model. Statistical significance was defined as a P value of less than 0.05. All analyses described above were performed with SPSS software (version 11, SPSS, Chicago, IL, USA). In this study, we investigated pretreatment predictive factors of early response, TTP, and survival in response to the combination therapy.

Results

Response to the combination therapy

The early response of the 55 patients was assessed after two courses of 5-FU/IFN combination therapy. As a result, 1 (2%), 15 (27%), 16 (29%), 12 (22%), and 11 (20%) patients showed CR, PR, SD, PD, or dropped out (DO), respectively. The reasons for DO were confusion (one patient), refusal after initiation of therapy (one patient), exanthema (one patient), infection around the catheter (four patients), and stenosis of the hepatic artery (four patients). We investigated the pretreatment determinants of the early response to the combination therapy. Univariate analysis identified positivity to HCV antibody as the only factor with significant influence on the early response (P = 0.028, Table 2, Fig. 1). Of the HCV antibody-positive patients, 38.9% (14/36) showed an early response of CR or PR, but only 10.5% (2/19) of other patients. When we compared the early response between patients with Vp 0–2 and those with Vp 3/4, 30.8% (8/26) of patients with Vp 0–2 and 27.6% (8/29) of those with Vp 3/4 achieved CR or PR, but the difference was not significant.

Time to progression

The median TTP in all 55 patients was 7.5 months [95% confidence interval (CI), 5.1–9.9 months], and the cumulative TTP rates at 6, 12, 18, and 24 months were 60%, 41%, 30%, and 24%, respectively. We investigated the pretreatment determinants of TTP after initiation of the combination therapy. Univariate analysis identified positivity for HCV antibody as the only factor with significant influence on TTP (P = 0.021, Table 3, Fig. 2). The median TTP in patients with Vp 0–2 and those with Vp 3/4 was 5.2 and 7.5 months, respectively. There was no significant difference in TTP between these two groups.

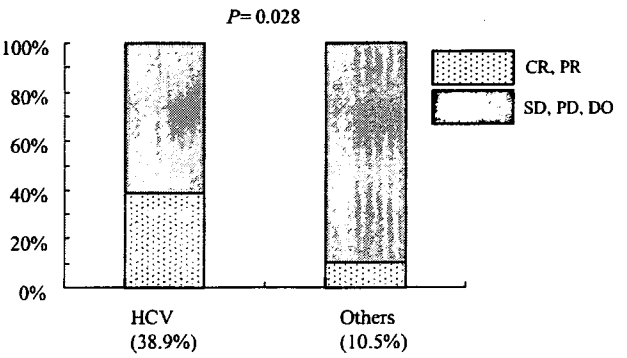

Fig. 1. Comparison of the early response rate between the hepatitis C virus (HCV)-positive group and others. The rate was significantly higher in the HCV-positive group (logistic regression test: $P = 0.028$). *CR*, complete response; *PR*, partial response; *SD*, stable disease; *PD*, progressive disease; *DO*, dropped out

Table 2. Univariate analysis of predictors for early response to 5-FU/IFN combination therapy

Variable	Odds Ratio	95% CI	P value
Age (≤65 vs. >65 years)	0.463	0.136–1.572	0.217
Sex (M vs. F)	2.327	0.445–12.168	0.317
HCV antibody (positive vs. negative)	6.071	1.216–30.314	0.028
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.931	0.240–3.614	0.918
Platelet count (≤150000 vs. >150000 mg/dl)	0.978	0.278–3.437	0.972
Albumin (≤3.5 vs. >3.5 mg/dl)	1.390	0.441–4.376	0.574
Child Pugh stage (A vs. B, C)	2.172	0.413–11.420	0.360
PS (0 vs. 1)	4.965	0.576–42.810	0.145
Intrahepatic tumor volume (≤50% vs. >50%)	1.690	0.458–6.237	0.431
Tumor stage (III vs. IVA)	1.709	0.533–5.478	0.367
Vp (0–2 vs. 3, 4)	0.988	0.314–3.106	0.983
AFP (≤10000 vs. >10000 ng/ml)	0.978	0.278–3.437	0.972
AFP-L3 (≤50 vs. >50%)	0.776	0.229–2.625	0.683
DCP (≤10000 vs. >10000 mAU/ml)	0.606	0.186–1.974	0.406
Treatment (performed vs. not performed)	1.833	0.563–5.970	0.314

5-FU, 5-fluorouracil; IFN, interferon; CI, confidence interval

Table 3. Univariate analysis of predictors of time to progression

Variable	Hazard Ratio	95% CI	*P* value
Age (>65 vs. ≤65 years)	1.348	0.177–10.263	0.773
Sex (M vs. F)	1.788	0.403–7.935	0.445
HCV antibody (positive vs. negative)	2.775	1.169–6.590	0.021
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.618	0.216–1.768	0.370
Platelet count (≤150000 vs. >150000 mg/dl)	0.739	0.307–1.777	0.500
Albumin (≤3.5 vs. >3.5 mg/dl)	0.705	0.300–1.655	0.421
Child Pugh stage (A vs. B, C)	2.381	0.314–18.045	0.401
PS (0 vs. 1)	1.348	0.177–10.263	0.773
Intrahepatic tumor volume (≤50% vs. >50%)	0.710	0.298–1.691	0.440
Tumor stage (III vs. IVA)	1.107	0.469–2.616	0.816
Vp (0–2 vs. 3, 4)	1.195	0.512–2.790	0.680
AFP (≤10000 vs. >10000 ng/ml)	1.325	0.484–3.626	0.584
AFP-L3 (≤50% vs. >50%)	2.371	0.696–8.076	0.167
DCP (≤10000 vs. >10000 mAU/ml)	1.145	0.486–2.701	0.756
Treatment (performed vs. not performed)	0.671	0.282–1.595	0.367

Table 4. Univariate analysis of predictors of survival of patients with HCC who received 5-FU/IFN combination therapy

Variable	Hazard Ratio	95% CI	*P* value
Age (≤65 vs. >65 years)	0.763	0.402–1.449	0.408
Sex (M vs. F)	1.208	0.527–2.769	0.655
HCV antibody (positive vs. negative)	2.283	1.165–4.474	0.016
Total bilirubin (≤1.5 vs. >1.5 mg/dl)	0.628	0.308–1.278	0.199
Platelet count (≤150000 vs. >150000 mg/dl)	0.690	0.355–1.340	0.273
Albumin (≤3.5 vs. >3.5 mg/dl)	0.760	0.398–1.451	0.406
Child Pugh stage (A vs. B, C)	0.527	0.228–1.216	0.133
PS (0 vs. 1)	3.413	1.391–8.375	0.007
Intrahepatic tumor volume (≤50% vs. >50%)	0.753	0.383–1.481	0.411
Tumor stage (III vs. IVA)	0.670	0.342–1.313	0.243
Vp (0–2 vs. 3, 4)	0.745	0.389–1.427	0.374
AFP (≤10000 vs. >10000 ng/ml)	0.947	0.445–2.017	0.888
AFP-L3 (≤50% vs. >50%)	0.898	0.430–1.871	0.773
DCP (≤10000 vs. >10000 mAU/ml)	0.753	0.394–1.438	0.390
Treatment (performed vs. not performed)	0.627	0.319–1.230	0.175
Additional therapy (performed vs. not performed)	1.129	0.583–2.188	0.719

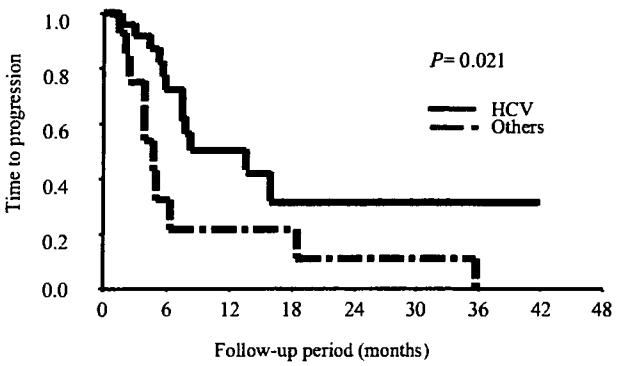

Fig. 2. Comparison of the time to progression between the HCV antibody-positive group and others. The rate was significantly higher in the HCV-positive group (log-rank test: $P = 0.021$)

Survival

The median survival in the whole group was 9.0 months (95% CI, 7.0–11.0 months), and the cumulative survival rates at 6, 12, 18, and 24 months were 67%, 39%, 22%, and 17%, respectively. We investigated the pretreatment determinants of survival after initiation of the 5-FU/IFN combination therapy. Univariate analysis identified PS = 0 ($P = 0.007$) and positivity for HCV antibody ($P = 0.016$) (Table 4, Fig. 3) as factors that significantly influenced survival. Since it was possible that the variables were mutually correlated, we performed a multivariate analysis and identified PS = 0 ($P = 0.003$) and positivity for HCV antibody ($P = 0.007$) as significant and independent determinants of survival (Table 5). The median survival time of patients with Vp 0–2 and of those with Vp 3/4 was 13.0 and 8.0 months, respectively. There was no significant difference in survival between these two groups.

Table 5. Multivariate analysis of predictors of survival of patients with HCC who received 5-FU/IFN combination therapy

Variable	Hazard Ratio	95% CI	*P* value
PS (0 vs. 1)	4.056	1.601–10.276	0.003
HCV antibody (positive vs. negative)	2.555	1.286–5.079	0.007

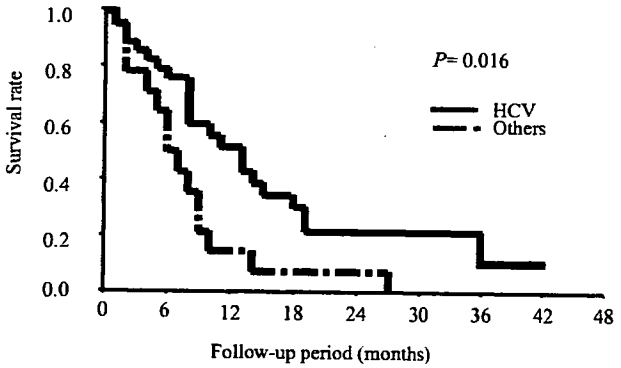

Fig. 3. Comparison of the cumulative survival rates between the HCV antibody-positive group and others. The rate was significantly higher in the HCV-positive group (log-rank test: $P = 0.016$)

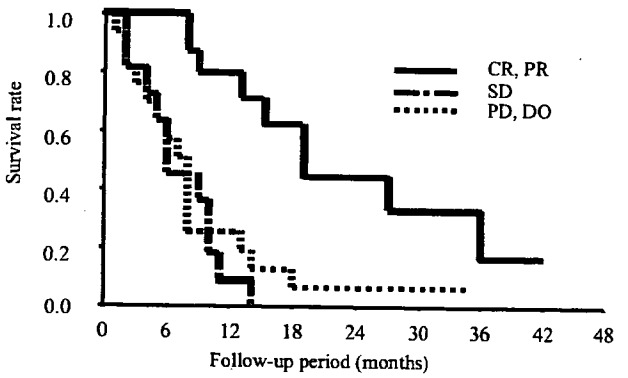

Fig. 4. Comparison of the cumulative survival rates among patients with CR/PR, SD, or PD/DO. The rate was significantly higher in patients who achieved CR/PR than those who showed SD (log-rank test: $P < 0.0001$) or PD/DO (log-rank test: $P < 0.0001$)

The cumulative survival rates of patients who achieved CR/PR at 6 and 12 months were 100% and 80%, respectively. On the other hand, the cumulative survival rates of patients who showed SD or PD/DO at 6 and 12 months were 64% and 9%, and 57% and 25%, respectively. The survival rate was significantly higher in patients who achieved CR/PR than in the other patients ($P < 0.0001$, Fig. 4).

Adverse reactions and complications

The most common adverse reactions were fever, nausea, and loss of appetite, but these were mostly NCI-CTC grade 1 or 2. Among patients with various NCI-CTC grade 3 adverse reactions, leukopenia was observed in seven (12.7%) patients, and thrombocytopenia in five (9.1%). None required administration of granulocyte colony-stimulating factor or blood transfusion. Five (9.1%) patients showed infection associated with the indwelling catheter. In this study, the number of patients with serum total bilirubin levels >3 mg/dl was three (3.7 mg/dl, 4.7 mg/dl, and 6.4 mg/dl). Other hepatic reserve functions and PS of the three patients was good (albumin, 4.1, 3.3, and 3.9 g/dl; prothrombin time, 60, 91, and 83%; PS, 0 in all cases). These three patients did not show any severe adverse reaction.

Additional therapy

Among the 55 patients, one (2%), ten (20%), and four (8%) patients were treated with RFA, TACE, and RT, respectively, as additional therapies for PVTT. The median survival time in patients receiving and in those not receiving additional therapies was the same at 9.0 months. There was no significant difference in survival between the two groups (Table 4).

Causes of death

Seventeen patients were still alive at the end of the observation period, and 38 patients had died. All 38 patients died of intrahepatic HCC-related disease.

Discussion

The median survival time of HCC patients with PVTT in the portal trunk is reported to be about 90 days with supportive care.[23] Recent studies have reported the efficacy and survival benefits of combination therapy with intraarterial 5-FU and IFN in a large number of patients with advanced HCC.[16,17] In particular, Ota et al.[16] assessed 55 patients with advanced HCC, multiple lesions, and Vp 3 or 4, and Obi et al.[17] assessed 116 patients with advanced HCC with Vp 3 or 4. These two studies assessed only patients with advanced HCC/

Vp 3 or 4. Thus, the favorable survival results they reported suggest that combination therapy with intra-arterial 5-FU and IFN is potentially useful also for HCC with Vp 0–2. Although TACE is the standard treatment option for nonresectable HCC, many patients with nonresectable HCC either show a poor response to TACE or are not suitable candidates for TACE. The prognosis of patients with nonresectable HCC who are not treated with TACE is poor, so an effective treatment for such patients is needed. In this study, we treated a heterogeneous group of patients with advanced HCC (i.e., patients with nonresectable HCC and Vp 3 or 4, those with nonresectable HCC and Vp 2 who were not suitable candidates for TACE, and those with nonresectable HCC without PVTT who showed a poor response to TACE). There was no significant difference in early response, TTP, or survival between HCC patients with Vp 0–2 and those with Vp 3/4. Hence, with regard to the response to 5-FU/IFN combination therapy, PVTT grade does not seem to be an important factor.

The objective response rates (CR and PR patients/all patients) reported in the above two studies[16,17] were 43.6% (24/55 patients) and 52.6% (61/116 patients). In our study, the objective response rate, based on the early response, was 29% (16/55 patients). One reason for the discrepancy may be that the response was evaluated differently in the three studies. Ota et al.[16] and Obi et al.[17] used ECOG criteria, but we used RECIST criteria. Second, the inclusion criteria were different. Ota et al.[16] included patients with aspartate aminotransferase (AST) or alanine aminotransferase (ALT) of less than 100 IU/l and patients with total bilirubin of less than 1.4 mg/dl, whereas Obi et al.[17] mentioned no inclusion criteria related to AST or ALT, though they used a total bilirubin level of 3.0 mg/dl as a cutoff. In our study, we used no inclusion criteria related to AST or ALT. Third, the assessment day in our protocol may be earlier than that in the other two studies. In our study, the early response cumulative survival rate was significantly higher in patients who achieved CR/PR than in those with SD or PD/DO ($P < 0.0001$, each). The early response is an important posttreatment predictor of survival of patients with advanced HCC on 5-FU/IFN combination therapy. An early response of CR or PR promises a good prognosis.

The cumulative survival rates reported by Ota et al.[16] and Obi et al.[17] at 12 and 24 months were 48.9% and 28.8%, and 34% and 18%, respectively. In our study, the cumulative survival rates at 12 and 24 months were 39% and 17%, respectively. We obtained survival rates almost identical to those reported by Obi et al.,[17] but Ota et al.[16] obtained better survival rates. This discrepancy may be due to the differences in the inclusion criteria, as described above.

Our results indicated that HCV antibody positivity was a significant pretreatment predictor of early response, TTP, and survival of patients with advanced HCC treated with 5-FU/IFN. On the other hand, PVTT grade and total bilirubin levels were not significant predictors. Though we established no eligibility criterion regarding serum total bilirubin levels, the median total bilirubin level was 1.1 mg/dl (range, 0.4–6.4). Therefore, total bilirubin levels may not be statistically significant predictors in this study. In this study, three patients had serum total bilirubin levels >3 mg/dl. These patient achieved PR, SD, and PD. Though the three patients with high bilirubinemia (≥3 mg/dl) were safely treated in this study, we think that 5-FU/IFN combination therapy should be used with caution in patients with advanced HCC with high bilirubinemia. In general, the prognosis of HCC patients with Vp 3 or 4 is poorer than those with Vp 0–2. In this study, we treated a heterogeneous group of patients with advanced HCC as described above. Therefore, HCC with Vp 0–2 cases were advanced HCC cases in this study. All 55 patients were thought to have a poor prognosis at the time of enrollment in this study. Achievement of a good early response is important for good survival. A study with larger sample size may show the importance of PVTT grade and total bilirubin level.

Obi et al.[17] also reported that positivity to HCV antibody might be a predictor of CR in patients with advanced HCC treated with 5-FU/IFN. Why is HCV antibody positivity a predictor of the efficacy of combination therapy? One reason may be the underlying mechanisms associated with hepatocarcinogenesis. In our study, 36 patients were infected with HCV, 15 with hepatitis B virus (HBV), and four with non-B non-C hepatitis. Although the probability of hepatocarcinogenesis is high for both HBV and HCV infections, some differences have been noted with regard to their relationship with HCC.[24,25] HCV is an RNA virus, and viral genes are not integrated into the host genome. On the other hand, HBV is a DNA virus with reverse-transcriptase activity. HBV-mediated hepatocarcinogenesis is reported to be associated with the integration of viral DNA into the host genome.[26–28] The integration of the HBV genome into the host genome may diminish the effect of intraarterial 5-FU/IFN combination therapy. A second reason may be the differentiation of the cytokine pattern in HBV and HCV hepatitis.[29] Falasca et al.[29] reported the presence of high levels of Th1 cytokines, particularly during the course of chronic hepatitis B. They also reported that interleukin (IL)-18 and IL-6 levels might play important roles in both inflammation and hepatic injury, particularly during the course of hepatitis C infection. IFN may play a different role in patients with advanced HCC associated with HBV or HCV. In this study, the efficacy of the

combination therapy for advanced HCC patients with non-B non-C hepatitis was not clear because of the small number ($n = 4$) of those patients.

The DO proportion was high in this study (20%). Two major reasons for DO were infection around the catheter and stenosis of the hepatic artery. In this study, we established no eligibility criterion regarding the hepatic reserve function, including serum total bilirubin. Poor hepatic reserve function and high bilirubinemia might affect infection around the catheter. On the other hand, previous treatment with TACE might injury the hepatic artery and affect hepatic artery stenosis.

In conclusion, HCV antibody positivity might be a pretreatment predictive factor for early response, TTP, and survival of patients with advanced HCC treated with intraarterial 5-FU/IFN combination therapy. Early response to the combination therapy might be a significant posttreatment predictor of survival. Thus, patients who do not achieve CR or PR during the early phase of combination therapy should be switched to another treatment modality. Our results also showed that PVTT grade does not seem to be an important factor in the prognosis of patients with advanced HCC treated with 5-FU/IFN combination therapy. Further studies with long-term follow-up and a larger sample size are needed.

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CLINICAL STUDIES

Similar effects of recombinant interferon-α-2b and natural interferon-α when combined with intra-arterial 5-fluorouracil for the treatment of advanced hepatocellular carcinoma

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Abstract

Aim: Intra-arterial 5-fluorouracil (5-FU) plus interferon (IFN) combination therapy is effective against advanced hepatocellular carcinoma (HCC) with portal vein tumour thrombosis. In this study, we compared the efficiency and safety of recombinant IFN-α-2b with natural IFN-α as components of the combination therapy. *Methods:* Consecutive HCC patients ($n=31$) with portal vein tumour thrombosis were enrolled in this prospective study. They received combination therapy of 5-FU and either recombinant IFN-α-2b (R group, $n=15$) or natural IFN-α (N group, $n=16$). We compared the two groups for the early response rate, adverse reactions, time to progression (TTP) and survival rates. In addition, we assessed the cost-effectiveness of each protocol. *Results:* The early response rate (R: 26.7%, N: 31.2%), median TTP (R: 5.8 months, N: 5.6 months) and median survival time (R: 7.5 months, N: 6.5 months) were not significantly different between the R and N groups. There were no differences in adverse reactions between the two groups. The estimated cost-effectiveness ratio of recombinant IFN-α-2b was better than natural IFN-α. *Conclusions:* In our protocol of combination therapy, there were no significant differences between recombinant IFN-α-2b and natural IFN-α with regard to early response to therapy, adverse effects, TTP and survival rates. 5-FU could be combined with either recombinant IFN-α-2b or natural IFN-α, although the cost-effectiveness of the former warrants its use clinically.

Hepatocellular carcinoma (HCC) is one of the most common neoplasms in Africa and Asia including Japan, and HCC-related deaths are increasing worldwide including Japan (1–3). Despite the progress in diagnostic techniques and therapeutic procedures, such as ultrasonography, computed tomography, magnetic resonance imaging, angiography, surgical resection, radiofrequency ablation (RFA), percutaneous ethanol injection (PEI) and transcatheter arterial chemoembolization (TACE), the prognosis of patients with HCC remains unsatisfactory. Furthermore, the survival rates of patients with advanced HCC and complications such as portal vein tumour thrombosis (PVTT) or distant metastasis remain extremely poor (4–8). PVTT frequently develops in HCC patients. In HCC patients with PVTT, tumour cells may spread out through the portal tract, resulting in intra-/extrahepatic metastases. Furthermore, portal vein occlusion may cause liver failure, ascites or variceal rupture. Thus, the performance status (PS) of HCC patients with PVTT gradually worsens, rendering them unsuitable for any treatment of HCC.

Advances in implantable drug-delivery systems have made it possible to administer repeated arterial infusions of anticancer agents. Recent studies reported the effectiveness of combination therapy of intra-arterial 5-fluorouracil (5-FU) and subcutaneous interferon (IFN)-α therapy for advanced HCC (9–11), with a response rate ranging from 47 to 73%. The majority of these studies used natural IFN-α. To our knowledge, there are no reports that have compared the effects of combination therapy of intra-arterial 5-FU and IFN

Table 1. Clinical profiles of the 31 patients with hepatocellular carcinoma

	Recombinant IFN-α-2b	Natural IFN-α	*P* value
N	15	16	
Age (years)	59 (26–79)	65(52–76)	NS
Sex (male/female)	12/3	14/2	NS
Grade of portal vein invasion (Vp 2/3/4)	2/3/10	1/8/7	NS
Main tumour size (mm)	52 (15–160)	57(25–140)	NS
Tumour volume (< 50%/≥ 50%)	9/6	10/6	NS
Child–Pugh grade (A/B/C)	10/5/0	13/3/0	NS
AFP (ng/mL)	3922.7 (51.2–708 100)	1957 (14.3–377 700)	NS
DCP (mAU/mL)	17 874 (< 10 – 233 450)	11 476 (46–722 140)	NS
Aetiology (HBV/HCV/others)	5/7/3	4/11/1	NS
Leucocyte count (/μL)	5790 (2210–8940)	6255 (2890–8910)	NS
Neutrophil count (/μL)	3395 (1260–6884)	4536 (1531–7008)	NS
Haemoglobin (g/dL)	12.5 (8.4–16.4)	12.9 (8.6–16.2)	NS
Platelet count (/μL) × 10^4	14.2 (5.1–54.4)	12.1 (5.9–34.2)	NS
Total bilirubin (mg/dL)	1.3 (0.6–2.7)	1.3 (0.7–2.8)	NS

Data are expressed as median values with ranges in parentheses, or number of patients. Portal invasion (Vp1, tumour thrombus in a third or more of the peripheral branches; Vp2, in the second branch; Vp3, in the first branch; Vp4, in the trunk).
AFP, α-fetoprotein; DCP, des-γ-carboxy prothrombin; HBV, hepatitis B virus; HCV, hepatitis C virus; IFN, interferon; NS, not significant.

with those of recombinant IFN-α-2b and natural IFN-α. Such studies are important because the activity of recombinant IFN-α-2b differs from that of natural IFN (12, 13), and the former is less expensive than natural IFN-α. Thus, if the safety and efficacy of recombinant IFN-α-2b is equal to or better than that of natural IFN-α, recombinant IFN-α-2b may be recommended with regard to cost-effectiveness. In the present prospective study, we investigated the safety of intra-arterial 5-FU and IFN and compared recombinant IFN-α-2b with natural IFN-α. In addition, we assessed the cost-effectiveness of each treatment regimen.

Materials and methods

Study design and eligibility

This was a prospective study conducted at our hospital to compare the outcome of recombinant IFN-α-2b and natural IFN-α in combination with intra-arterial 5-FU. The eligibility criteria were as follows: age (18–80 years), Child–Pugh status A or B, leucocyte count > 2000/μL, neutrophil count > 1200/μL, haemoglobin > 8 g/dL, platelet count > 50 000/μL, total bilirubin < 3.0 mg/dL, serum creatinine < 1.5 mg/dL, unresectable or not suitable for local ablation therapy, main tumour size > 20 mm, tumour number > 2, presence of PVTT (in the second branch first branch or trunk), an Eastern Cooperative Oncology Group PS of 0–1 (14) and without extrahepatic metastases. All patients were asked to give their written informed consent to this study, which was approved by the Institutional Review Board of Hiroshima University.

From June 2003 to December 2006, 265 consecutive patients with unresectable HCC were admitted to our hospital. As a result of the progression of HCC (e.g. PVTT, extrahepatic metastases), these patients were not suitable candidates for either surgical resection or local ablation therapy, including RFA and PEI. Of the 265 patients with advanced HCC, 39 were considered to be suitable candidates for the intra-arterial 5-FU and IFN combination therapy. Eight patients could not be included because of refusal of enrolment. Thus, 31 patients with advanced HCC without extrahepatic metastases were enrolled in this prospective study. We treated the first 15 consecutive patients with recombinant IFN-α-2b (R group) and the second 16 consecutive patients with natural IFN-α (N group), combined with 5-FU.

Table 1 lists the baseline characteristics of the patients of the two groups (R group vs. N group). There were no differences between the two groups with respect to the sex ratio, age, proportion of patients with hepatitis B virus and hepatitis C virus infection, PVTT in the second branch (Vp2), major branch (Vp 3) and main trunk (Vp 4), median level of α-fetoprotein and des-γ-carboxy prothrombin, median leucocyte count, neutrophil count, haemoglobin, platelet count, total bilirubin and duration of the observation period [R: 10.5 (0.7–39.4 months), N: 6.3 (1.1–16.7 months)].

Treatment protocol

Patients received repeated arterial infusions of anticancer agents via an injection port. One course of

chemotherapy represented 4 weeks. 5-FU (500 mg/body weight/day; Kyowa Hakko, Tokyo, Japan) was administered within 5 h using a mechanical infusion pump on days 1–5 of the first and second weeks (5 g in one course). Recombinant IFN-α-2b (Intron A®; Schering-Plough Pharmaceuticals Co., Osaka, Japan) at 3×10^6 U (3 MU), or natural IFN-α (OIF®; Otsuka Pharmaceuticals Co., Tokyo, Japan) at 5×10^6 U (5 MU) was administered intramuscularly on days 1, 3 and 5 of each week (total dose of 36 and 60 MU respectively). In our hospitals, the minimum dose of recombinant IFN-α-2b for the treatment of chronic hepatitis C is 3×10^6 U and that of natural IFN-α is 5×10^6 U. Previous reports used 5×10^6 U as the minimum dose of natural IFN-α. With regard to recombinant IFN-α-2, we selected the above dose as the minimum dose in order to avoid potential adverse effects. In principle, treatment was repeated several courses unless PS changed to 3 or 4 during the treatment. A 2–4-week rest period of no treatment was allowed after each treatment course.

Implantation of arterial catheter

The catheter was inserted through the right femoral artery using the Seldinger method. After the detection of HCC, a 3-French heparin-coated catheter (Clinical Supply, Gifu, Japan) was inserted and its tip was advanced to the common hepatic artery or proper hepatic artery. The other end of the catheter was connected to the injection port, which was implanted in a subcutaneous pocket created in the right lower abdominal quadrant. The gastroduodenal and right gastric arteries were occluded using steel coils to prevent potential gastroduodenal injury from the anticancer agents.

Evaluation

The response to treatment was assessed in all patients enrolled in this study. The response was defined according to the criteria of the Response Evaluation Criteria in Solid Tumors (RECIST) (15). A complete response (CR) was defined as the complete disappearance of all target lesions. A partial response (PR) was defined as at least a 30% decrease in the sum of the longest diameter (LD) of target lesions with the baseline sum of the LD of target lesions as the reference. Progressive disease (PD) was defined as at least a 20% increase in the sum of the LD of target lesions. Stable disease (SD) was defined as neither PR nor PD criteria fulfilled. The duration of response was measured from the date of the start of treatment to the date of documented progression.

Adverse reactions were assessed every week during the treatment using the National Cancer Institute Common Toxicity Criteria (NCI-CTC) (version 3.0) (16).

Cost-effectiveness

The cost of each IFN with two courses of treatment was calculated. The effectiveness of treatment was reflected by the percentages of patients who achieved CR or PR. The cost-effectiveness ratio was calculated using the formula: cost/effectiveness.

Additional therapy

After two courses of the combination therapy, we assessed the response to therapy in all patients. According to the response, we provided various additional therapies such as RFA, TACE and radiotherapy (RT) for patients treated with the combination therapy. Patients assessed as PR continued to receive the combination therapy, in addition to local ablation therapy when further decrease of HCC was not expected. All patients assessed as SD or PD received RT for PVTT. Furthermore, one session of TACE using a cisplatin–lipiodol suspension was repeated before the initiation of each course of intra-arterial 5-FU and IFN combination therapy unless the Child–Pugh status changed to C.

Statistical analysis

Statistical analysis was performed on 31 December 2006. Differences between groups were examined for statistical significance using the Mann–Whitney test (*U*-test) and χ^2 test where appropriate. Cumulative survival rate and time to progression (TTP) were assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log-rank test. All analyses described above were performed using the SPSS program (version 11; SPSS Inc., Chicago, IL, USA).

Results

Early response rate

We assessed all patients after two courses of treatment. Of all 31 patients, one (3.2%), eight (25.8%), eight (25.8%), 10 (32.3%) and four (12.9%) patients showed CR, PR, SD, PD and drop-out (DO) respectively (Table 2). For the R group, zero (0%), four (26.7%), three (20%), five (33.3%) and three (20%) patients showed CR, PR, SD, PD and DO respectively. The reasons for DO were confusion (one patient), refusal after initiation of therapy (one patient) and exanthema (one patient). The overall response rate

Table 2. Response to treatment after two courses

	CR	PR	SD	PD	DO	Response rate*
Recombinant IFN-α-2b ($n = 15$)	0	4	3	5	3	26.7%
Natural IFN-α ($n = 16$)	1	4	5	5	1	31.2%

*Response rate = CR + PR/CR + PR + SD + PD.
CR, complete response; DO, drop out; IFN, interferon; PD, progressive disease; PR, partial response; SD, stable disease.

was 26.7% for the R group. For the N group, one (6.3%), four (25%), five (31.2%), five (31.2%) and one (6.3%) patients showed CR, PR, SD, PD and DO. The reason for DO was infection around the catheter (one patient). The overall response rate for the N group was 31.2%. There was no statistically significant difference in the early response between the two groups.

Adverse reactions and complications

Table 3 summarizes the adverse reactions and complications encountered during and after the treatment. The most common adverse reactions were fever, nausea and loss of appetite, but these were mostly NCI-CTC Grade 1 or 2. The percentages of patients with various NCI-CTC Grade 3 adverse reactions of the two treatment groups were not significantly different. With regard to the two patients of the R group with leucopenia, the initial leucocyte counts were 2210 and 3980/μL and the lowest counts were 1214 and 1524/μL respectively. As for the four patients with thrombocytopenia, the initial platelet counts were 61 000, 114 000, 185 000 and 227 000/μL, and the lowest counts were 31 000, 48 000, 45 000 and 37 000/μL respectively. None required administration of granulocyte-colony-stimulating factor (G-CSF) or blood transfusion, and none developed depression. Complications associated with the indwelling catheter were infection (one patient each).

Additional therapy after two courses of combination treatment

For the R group, four patients received TACE, one patient received RT and two patients received both TACE and RT. In the four patients who achieved PR, three continued to receive the combination therapy while the fourth achieved CR after completing four courses of the combination therapy, and undergoing RFA for the remaining HCC. With regard to the N group, four patients received TACE, two patients underwent RT and one patient received both TACE and RT. With regard to the four patients who achieved PR, three continued the combination therapy, while the fourth patient achieved CR after receiving three courses of the combination therapy, followed by RT for PVTT.

Table 3. Adverse reactions (National Cancer Institute Common Toxicity Criteria Grade 3) and complications during and after the combination treatment

	Recombinant IFN-α-2b	Natural IFN-α	*P* value
Leucopenia	2 (13.3%)	0	NS
Thrombocytopenia	1 (6.7%)	3 (18.8%)	NS
Nausea	1 (6.7%)	0	NS
Exanthema	1 (6.7%)	0	NS
Confusion	1 (6.7%)	0	NS
Infection around the catheter	1 (6.7%)	1 (6.3%)	NS
Pseudoaneurysm of the femoral artery	1 (6.7%)	1 (6.3%)	NS

IFN, interferon; NS, not significant.

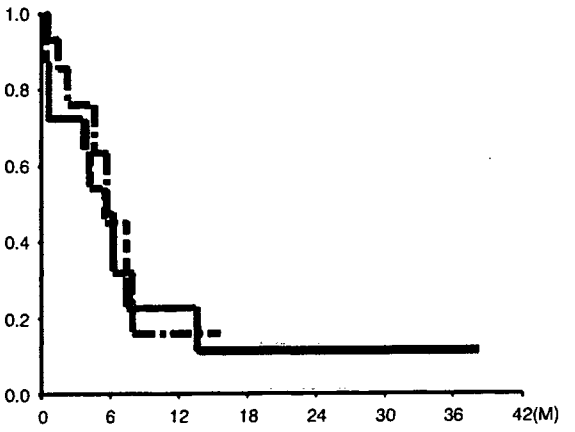

Fig. 1. Comparison of the time to progression of patients with hepatocellular carcinoma complicated with portal vein tumour thrombosis and treated with 5-fluorouracil (5-FU) and recombinant interferon (IFN)-α-2b (solid line) or with 5-FU combined with natural IFN-α (log-rank test: not significant).

Time to progression

The median TTP for all 31 patients was 5.8 months [95% confidence interval (CI), 3.9–7.7 months]. For the R and N groups, the median TTP was 5.6 months (95% CI, 3.0–8.2 months) and 5.8 months (95% CI,

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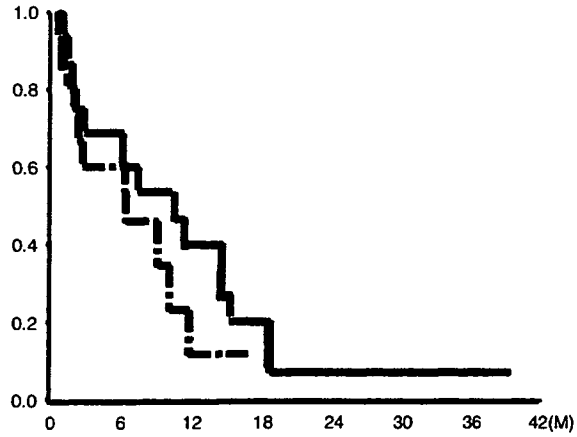

Fig. 2. Comparison of the survival rate of patients with hepatocellular carcinoma complicated with portal vein tumour thrombosis and treated with 5-fluorouracil (5-FU) and recombinant interferon (IFN)-α-2b (solid line) or with 5-FU combined with natural IFN-α (log-rank test: not significant).

4.0–7.6 months) respectively. There was no significant difference in TTP between the two groups (Fig. 1).

Survival rates

Of all 31 patients, the median survival time was 7.5 months (95% CI, 3.3–11.7 months) and the cumulative survival rates at 6, 12, 18 and 24 months were 64.5, 29.0, 16.5 and 5.6% respectively. The median survival time of the R group [10.5 months (95% CI, 0.0–21.4 months)] was not significantly different from that of the N group [6.5 months (95% CI, 3.7–9.3 months)] (Fig. 2).

Cost-effectiveness

The cost of IFN with two course of treatment was $US1052 for the R group and $US3060 for the N group. Four (26.7%) patients of the R group showed PR, while one (6.3%) and four (25%) patients of the N group showed CR and PR respectively. Thus, the estimated effectiveness was 26.7 for the R group and 31.3 for the N group. The cost-effectiveness ratios were 39.4 for the R group and 97.8 for the N groups, indicating that the R combination therapy was about three times more cost-efficient than the N combination therapy (Table 4).

Causes of death

Seven patients were still alive at the end of the observation period while 24 patients had died. All the 24 patients died of cancer-related disease.

Table 4. Cost-effectiveness of each interferon with two courses of the treatment

	Cost ($)	Effectiveness* (%)	Cost-effectiveness ratio†
Recombinant IFN-α-2b	1052	26.7	39.4
Natural IFN-α	3060	31.3	97.8

*Effectiveness; percentage of patients who showed CR or PR.
†Cost-effectiveness ratio = cost/effectiveness.
CR, complete response; IFN, interferon; PR, partial response.

Discussion

The prognosis of patients with advanced HCC complicated with PVTT remains poor, particularly in those patients with PVTT in the first branches or the portal trunk. The median survival time of HCC patients with PVTT in the portal trunk is reported to be about 90 days with supportive care (17). In this regard, Patt *et al.* (18) reported the efficiency of intravenous 5-FU combined with recombinant IFN-α-2b. Recently, several studies assessed the efficacy of combination therapy of intra-arterial 5-FU and IFN (9–11). However, most of the reports that analysed the effects of combination therapy of intra-arterial 5-FU and IFN used natural IFN-α. The use of recombinant IFN-α-2b has been reported in only one study (9). The response rates (CR and PR patients/all patients) reported in the two studies by Sakon *et al.* (10) and Obi *et al.* (11) were 73% (8/11 patients) and 52.6% (61/116 patients) respectively. In this study, for patients of the two groups, the objective response rate according to the early response was 29% (9/31 patients). The discrepancy between the studies may be because of the following reasons. First, the early response to our protocol was assessed after two courses of the treatment, while others evaluated the maximum response. Second, the method of evaluation of the response was different. The above two studies used the Eastern Cooperative Oncology Group criteria but we used the RECIST criteria. Third, the sample size was very small in the report by Sakon *et al.* (10). In our study, the survival rates of the patients of the two groups were almost identical to those reported by Obi *et al.* (11) (the survival rates at 6 and 12 months were 53 and 34% respectively). Thus, the protocol used in our study was considered to be suitable for patients with advanced HCC.

Both IFN-α and IFN-β induce the transcription of the p53 gene and contribute in boosting the responses to p53 activation, which suppresses cancer (19). IFN-α is also known to inhibit cancer cells directly as well as indirectly (20–26) and to have anti-angiogenic and

antiviral activities. The direct antineoplastic effects include cell damage (27), induction of cyclin-dependent kinase inhibitors involved in G1/G0 arrest (22) and delayed cell cycle (28). The indirect antineoplastic effects include activation of natural killer cells, T cells and macrophages (29–31). In various cultures of malignant cells, IFN-α exhibited a biomodulatory effect that enhanced the antineoplastic activity of 5-FU partly because of the arrangement of metabolism of 5-FU to fluoro-deoxy-uridylate (32–36). Furthermore, 5-FU and IFN-α synergize the antineoplastic effects of each other. The antineoplastic effects of the combination therapy of intra-arterial 5-FU and IFN are also considered to be mediated by modulating tumour necrosis factor-related apoptosis-inducing ligand receptor-induced cytotoxic pathway (37).

Several subtypes of natural IFN-α have been described (38), while only one subtype is available for recombinant IFN-α. Patients treated with natural IFN-α barely have antibodies to IFN, whereas circulating antibodies to IFN are sometimes detected in patients treated with recombinant IFN-α (12, 13). Antibodies to IFN weaken the therapeutic effects of IFN. Therefore, antibodies to IFN may dampen the effects of the combination therapy of intra-arterial 5-FU and IFN. This hypothesis favours the combination therapy of intra-arterial 5-FU and natural IFN-α relative to 5-FU and recombinant IFN-α.

Interferon-α subtypes exhibit several variations in biological activity. With regard to the antiviral activity, IFN-α8 is reported to be the most potent while IFN-α1 the least potent (39). IFN-α8 was the most potent in the induction of antineoplastic effect on renal cell carcinoma (40). OIF®, but not Intron A®, contains IFN-α8. Considered with the deficiency of the subtypes *in vitro*, the effect of combination therapy with IFN-α may be different based on the IFN.

Our results, however, showed no significant differences between the two groups with respect to the early response, adverse reactions, TTP and survival rate. What are the reasons for the lack of differences *in vivo*? One reason may relate to the dose and antineoplastic activity of IFN (19–31, 39, 40). Several groups have studied the impact of IFN treatment on HCC. Two controlled trials reported by Lai *et al.* (41, 42) using very high doses of IFN (50×10^6 IU/m^2) showed a 30% response rate and improvement in survival compared with no treatment. In comparison, another study using a low dose of IFN (3×10^6 IU/m^2) did not show any survival advantage (43). Considered together, it appears that for IFN alone to be effective against HCC, its dose must be higher than that used for the treatment of chronic hepatitis B and C. Administration of high-dose IFN may improve the effect of the combination therapy. However, under such circumstances, many patients could potentially DO because of the adverse reactions. Thus, our protocol is safe regardless of the type of IFN used. The second reason relates to the relationship between IFN subtype and the mechanism of action of the combination therapy (32–40). Although the mechanism is not yet clear, the direct effect of inhibition of cancer cells and the anti-angiogenic effect of IFN might play minor roles in our protocol *in vivo*. The most important mechanism of action of the combination therapy of our protocol may be enhancement of the antineoplastic effect of 5-FU by IFN. Thus, the IFN subtype does not seem to strongly influence the effect of the combination therapy. The third reason may relate to the several limitations in our study (e.g. small sample size, not randomized-controlled trial).

Most of the adverse reactions were controllable in the present cohort. The adverse reactions of anaemia, leucopenia and thrombocytopenia were controllable without G-CSF or blood transfusion. Depression owing to IFN was not observed in our patients. Thus, the lack of severe pancytopenia in patients with advanced HCC treated by the current protocol reflects the safety of intra-arterial 5-FU and IFN. It is recommended, however, that careful treatment should be provided to patients who develop pancytopenia.

Our study fell somewhat short of conclusiveness owing to the small number of patients. Thus, our study should be extended to include a long-term follow-up and a large sample size. In our protocol of the combination therapy, there were no significant differences between recombinant IFN-α-2b and natural IFN-α with regard to early response to therapy, adverse effects, TTP and survival rates. Recombinant IFN-α-2b is inexpensive compared with natural IFN-α. Our analysis showed a better cost-effectiveness ratio for recombinant IFN-α-2b than natural IFN-α. Thus, assuming no difference in outcomes between the two regimens, we recommend the use of recombinant IFN-α-2b based on the cost-effectiveness.

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Biliary Complications after Duct-to-duct Biliary Reconstruction in Living-donor Liver Transplantation: Causes and Treatment

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Abstract
Background In living-donor liver transplantation (LDLT), biliary complications are recognized as a significant cause of post-transplantation morbidity.
Methods Eighty patients who underwent LDLT with duct-to-duct biliary reconstruction at Hiroshima University Hospital were enrolled in this study. The mean follow-up was 24 months (range, 3–72 months). Eighteen patients underwent the basiliximab-based immunosuppressive therapy, and 62 patients underwent non-basiliximab-based immunosuppressive therapy. The development of biliary complications after LDLT was retrospectively analyzed. Biliary complications were initially treated by endoscopic or radiological modalities.
Results Biliary leakages and strictures occurred in 12 (15%) and 20 (25%) of the 80 patients, respectively. Stepwise multivariate analysis demonstrated bile leakage to be an independent risk factor for the development of biliary stricture ($p = 0.001$) and basiliximab-based immunosuppressive therapy to be an independent protective factor for postoperative biliary leakage ($p = 0.005$). The 1-week total doses of steroids were significantly lower in the basiliximab-based immunosuppressive regimes (mean dose: 573mg) than in the non-basiliximab-based ones (mean dose: 1,121mg) ($p = 0.01$). All patients with biliary leakage were successfully treated with endoscopic or radiological modalities, except one patient who was treated by surgical treatment. Endoscopic or radiological modalities were successful as primary treatment modalities in 12 (60%) of 20 patients with biliary strictures. Lastly, six patients were treated surgically with long-term success, except for one patient with chronic cholangitis who died after 16 months.
Conclusions Steroid-sparing basiliximab-based immunosuppressive therapy reduced the incidence of biliary leakage, and biliary leakage was the independent factor for biliary stricture. The non-surgical and surgical treatments for biliary complications were satisfactory.

Various refinements in surgical techniques, postoperative management, and immunosuppressive management have reduced the incidence of complications after liver transplantation. Biliary complications, however, continue to be a significant cause of morbidity after liver transplantation [1, 2]. In living-donor liver transplantation (LDLT), the biliary system is usually reconstructed by performing a Roux-en-Y hepaticojejunostomy (RYHJ), which results in biliary complications in 12%-18% of recipients [3, 4]. In 1998, Wachs et al. first reported duct-to-duct reconstruction for LDLT [5]. Duct-to-duct direct biliary reconstruction has been performed in many institutes, and the advantages of duct-to-duct biliary reconstruction over hepaticojejunostomy have been pointed out in several reports. For example, it preserves the physiological bilioenteric and bowel continuity, thus preventing delayed bowel movements. Further, it permits easy endoscopic access to the biliary tree for diagnostic and therapeutic instrumentation and assists the prevention and management

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of ascending cholangitis [6–9]. As the number of patients who have undergone LDLT with duct-to-duct biliary reconstruction has increased, however, a variety of biliary complications have emerged [10–12]. Some reports have addressed the occurrence of late biliary complications, particularly biliary strictures, in many patients with a significant median follow-up duration (>12 months) [9, 13]. In the present study, to evaluate the safety of duct-to-duct biliary reconstruction in LDLT, we retrospectively analyzed the biliary complications observed, with focus on biliary leakage and stricture.

Materials and methods

Patients and surgical procedures

Between May 2000 and September 2006, 85 patients underwent LDLT at Hiroshima University. Among these patients, the 80 patients who underwent duct-to-duct biliary reconstruction along with LDLT were enrolled in this study. Patient, graft, and operative characteristics are summarized in Table 1. The series comprised 47 men and 33 women (average age: 50 years). The most common indications for LDLT were viral hepatitis and cirrhosis with or without hepatocellular carcinoma (n = 54), followed by fulminant hepatic failure (n = 10), primary biliary cirrhosis (n = 8), autoimmune hepatitis (n = 6), and others (n = 2). The most commonly used graft type was a right hemi-liver (n = 66), followed by a left hemi-liver (n = 14). The donors included 49 men and 31 women (average age: 35 years).

The mean model for end-stage liver diseases (MELD) score at the time of LDLT was 17.9 (range: 5–50). The mean graft-to-recipient weight ratio (GRWR) was 1.08 (range: 0.5–2.4); moreover, none of the grafts included a middle hepatic vein. The mean operative time was 12 h 8 min (range: 9–25 h). The mean total ischemic time was 108 min (range: 43–240 min), and the warm ischemic time was 45 min (range: 32–220 min).

The immunosuppressive regimen comprised cyclosporine with mycophenolate mofetil (MMF) and methylprednisolone and basiliximab (n = 18), or cyclosporine with MMF and methylprednisolone (n = 8), or tacrolimus with methylprednisolone (n = 56). The steroid-sparing basiliximab-based immuosuppressive therapy was indicated for viral hepatitis, because the steroid might contribute to the acceleration of hepatitis viral replication. Basiliximab 20 mg was given intravenously on both day 0 and day 4 after surgery. Tacrolimus was administered with a level of 5 ng/ml for the first 48 h postoperatively in order to maintain renal function. Then, the dose of tacrolimus was adjusted to maintain a level of 10–15 ng/ml during the first month, and afterward tapered to achieve a level of 5–10 ng/ml. Cyclosporine was also administered at a level of 50–100 ng/ml for the first 48 h postoperatively in order to maintain renal function. Then, the dose of cyclosporine was adjusted to maintain a level of 250–300 ng/ml during the first month, after which it was tapered to achieve a level of 150–250 ng/ml. Dose reductions of both tacrolimus and cyclosporine were performed primarily on the basis of renal and liver function. For patients with renal insufficiency, tacrolimus or cyclosporine was not given until renal function improved. After oral medication capsules were tolerated, MMF was given at a dose of 500–1,000 mg a day. Mycophenolate mofetil was tapered and discontinued, based on gastrointestinal toxicity and myelosuppression. Treatment with steroids was discontinued 2–3 months after LDLT. In basiliximab-based immuosuppressive therapy, patients either received no methylpredonisolone or they received 250 mg methylpredonisolone intravenously during surgery, followed by daily tapering (starting at 120 mg/day and ending at a baseline 40 mg/day, intravenously).

Table 1 Living-donor liver transplantation patient demographics

Characteristics	Number = 80
Age (range, years)	50 (20–69)
Male	47 (59%)
MELD (range)	17.9 (5–50)
Indication	
Liver cirrhosis (HCC)	54 (35)
Cholestatic disease	8
Fulminant hepatic failure	10
Autoimmune hepatitis	6
Others	2
Donor	
Age (range, years)	35 (18–64)
Male	49 (31)
Graft	
Left lobe	14
Right lobe	66
GRWR	1.08 (0.5–2.4)
Immunosuppresive therapy	
Tac + steroid	54
CyA + steroids + MMF	8
CyA + steroids + MMF + Bax	18
Operation	
Time (range)	12 h 8 min (9h–25h)
Blood loss (range)	4875ml (345–39500)
Total ischmic time (range)	108min (43–240)
Warm ischmic time (range)	45min (32–220)

MELD model for end-stage liver disease; GRWR graft: recipient weight ratio; Tac tacrolimus; CyA cyclosporin; MMF mycophenolate mofetil; Bax basiliximab

Treatment with oral methylpredonisone (32 mg/day) was initiated on postoperative day 7–10. In non-basiliximab based immunosuppression therapy, patients received 500 mg methylpredonisolone intravenously during surgery, followed by daily taper (starting at 250 mg/day and ending at a baseline 40 mg/day, intravenously). Treatment with oral methylpredonisone (32 mg/day) was initiated on day 7–10. Subsequent adjustment in maintenance methylpredonisolone was dependent on the patient's clinical course.

Donor assessment and surgery

The donors underwent several preoperative examinations, including computed tomography (CT) and drip-infusion cholangiography-CT, in order to assess the biliary and vascular system. The surgical techniques for donor hepatectomy have been described elsewhere [14]. Briefly, prior to parenchymal transection, routine intraoperative cholangiography was performed with fluoroscopy to determine the transection point of the hepatic duct. Minimal dissection was performed at the hilar plate around the hepatic duct. The liver was then transected with an ultrasonic dissector without inflow occlusion. The hepatic duct was sharply severed near the confluence, and the remnant stump was carefully closed with 6–0 polydioxanone monofilament sutures (PDS; Ethicon, Inc., Tokyo, Japan). The liver graft was perfused with University of Wisconsin (UW) solution. The diameters of the bile duct and vessels of the graft and the graft weight were directly measured. The average intraoperative blood loss was 310 ml. None of the 80 donors were given a blood transfusion.

Recipient surgery

In total hepatectomy, the hilar plate was dissected sharply at or distal to the second-order branch of the bile duct. In the dissection, careful attention was paid in order to preserve as much as possible of the surrounding tissues with an adequate blood supply to the bile duct. To maintain the blood supply to the bile duct from the right hepatic artery, dissection between the right hepatic artery and the bile duct was avoided. Bile duct anastomosis was performed after completion of all vascular anastomoses and reperfusion of the liver graft. Wherever possible, we prospectively performed duct-to-duct biliary reconstruction. An end-to-end anastomosis between the graft and recipient bile ducts was performed using an interrupted 6–0 PDS, beginning from the posterior wall and terminating at the anterior wall. In the case of more than one ductal opening in the graft, if the openings were adjacent to each other, ductoplasty was performed to suture them to form a single orifice. If two ductal openings in the graft were far apart, separate duct-to-duct anastomoses were performed without ductoplasty. A stent tube was routinely placed through the anastomosis as a splint and was pulled out through the common bile duct above the duodenum. A cholangiogram was obtained by using the inserted stent tube 1 month after LDLT, and then the stent tube was clamped. The tubes for bile duct stenting were removed 3 months after LDLT.

Diagnosis and treatment of biliary complications

Biliary leakage was diagnosed clinically and radiologically on the basis of a bile leak through abdominal drains, evacuation of extrahepatic biloma through a newly inserted drain under ultrasound guidance, or identification of a leak by endoscopic retrograde cholangiography (ERC) or cholangiography via an inserted stent tube. For biliary leakage, endoscopic retrograde nasobiliary drainage (ENBD) or percutaneous drainage under ultrasound guidance were the techniques most commonly undertaken.

Biliary stricture is primarily suspected when cholestatic enzymes that are assessed by liver function tests, including alkaline phosphatase and γ-glutamyl transpeptidase, are elevated and/or if there is sonographic evidence of a dilated biliary system. If the total bilirubin was not elevated, drip-infusion cholangiography-CT was performed. The presence of strictures was confirmed by ERC and/or percutaneous transhepatic cholangiography (PTC). Biliary stenosis was diagnosed on the basis of an abrupt luminal narrowing with an overt dilatation of the intrahepatic duct.

Primary transpapillary intervention was attempted in all patients who underwent duct-to-duct biliary reconstruction. Endoscopic retrograde balloon cholangioplasty was performed; this was followed by the placement of a plastic internal stent tube. When endoscopic treatment failed, percutaneous management of the biliary stricture was undertaken. Surgical revision was indicated when both these modalities failed.

Statistical analysis

Category variables were compared with the chi-square test. Continuous data were compared by the Mann-Whitney test. Patient survival after liver transplantation was analyzed by the Kaplan-Meier survival method. The statistical comparison of survival data was performed with the log-rank test. Stepwise logistic regression analysis was carried out in order to identify the independent predictors of biliary complications. A p value < 0.05 was considered to be significant. All statistical analyses were performed with the statistical software package SPSS version 11.0 (SPSS Inc. Chicago, IL).

Results

Type of biliary reconstruction

Table 2 shows the type of bile duct reconstruction with the corresponding incidence rate of biliary stricture. Forty-eight (60%) grafts had a single duct for anastomosis, 29 grafts (36%) had two ducts, and 3 grafts (4%) had three ducts. After ductoplasty in 14 grafts, 62 grafts had a single duct for anastomosis, 15 grafts had two ducts for anastomosis, 2 grafts had three ducts for anastomosis, and 1 graft had two ducts for anastomosis.

Overall incidence of biliary complications, risk factors, and outcomes after LDLT

Biliary leaks developed in 12 patients (15%), and 20 (25%) of the 80 patients suffered from a post-transplantation biliary stricture (Table 2). The mean follow-up was 24 months (range: 3–72 months). The onset of biliary leakage was 20 ± 8 days. No patient developed a de novo biliary stricture beyond 20 months after LDLT. Seven patients (8.8%) developed both the biliary complications. None of the five patients that underwent hepaticojejunostomy developed a biliary stricture. Further, there were no hepatic arterial complications in our series. By univariate analysis, we found two variables to be associated with an increased risk of biliary stricture: a postoperative bile leakage and non-basiliximab-based immunosuppressive therapy (Table 3). After stepwise multivariate analysis, one variable remained significant, i.e., postoperative bile leakage ($p = 0.001$) (Table 3). There were no significant differences in the incidence of biliary stricture with respect to donor age, MELD score, graft type, the number of bile ducts, and ductoplasty. There was no significant difference in the incidence of biliary stricture with respect to the number and mode of anastomotic sutures. However, in the grafts that had three ducts, we observed a high incidence of biliary stricture (2/3, 66.6%) (Table 2). We next examined the incidence of biliary stricture according to the diameter of the anastomosis. Graft duct sizes were classified into small (diameter <4 mm), medium (diameter 4–5 mm), and large (diameter >5 mm). Recipients with two or three biliary ducts were excluded in order to avoid bias from complex biliary reconstructions. We observed no association between the diameter of the bile ducts and the incidence of anastomotic suture.

Interestingly, both biliary leaks and strictures developed less frequently in patients with basiliximab-based immunosuppressive regimes. In stepwise multivariate analysis, non-basiliximab-based immunosuppressive therapy was associated with an increased risk for postoperative bile leakage ($p = 0.005$) (Table 4). Further, we found that the 1-week doses of methylpredonisolone after LDLT were significantly lower in basiliximab-based immunosuppressive regimes than in non-basiliximab-based ones ($p = 0.01$) (Fig. 1).

Freedom from biliary stricture was 73% at 1 year and 69% at 2 years (Fig. 2). The 1-year and 5-year survival rates for patients with biliary stricture were 69% and 53%, respectively, compared with 79% and 70% for those without biliary strictures ($p = 0.31$) (Fig. 3).

Table 2 Biliary complications after duct-to-duct biliary reconstruction in living donor liver transplantation

	n	Leakage (%)	stricture (%)
Number of bile ducts and anastomoses	80	12 (15)	20 (25)
1 duct / 1 anastomoses	48	5 (10.4)	11 (22.9)
2 ducts / 1 anastomoses (plasty)	14	3 (21.4)	5 (35.7)
2 ducts / 2 anastomoses	15	2 (13.3)	2 (13.3)
3 ducts / 3 anastomoses	2	1 (50)	2 (100)
3 ducts / 2 anastomoses (plasty)	1	0	0

Table 3 Univariate and multivariate analysis of risk factors for biliary strictures

Risk factors	No. of patients with biliary stricture	*p* Value (Univariate)	*p* Value (Multivariate)
Immunosuppression		0.044	0.124
Bax (*n*=18)	1 (5%)		
Non-Bax (*n*=62)	19 (30.6%)		
Biliary leakage		0.0001	0.001
Yes (*n*=12)	7 (58%)		
No (*n*=68)	13 (19%)		
No. of bile ducts		0.501	0.165
Single (*n*=48)	11 (22.9%)		
Non-single (*n*=32)	9 (28.1%)		
Ductoplasty		0.216	0.121
Yes (*n*=15)	5 (33.3%)		
No (*n*=65)	15 (23.1%)		
Donor age		0.072	0.152
> 50 year (*n*=28)	10 (35.7%)		
< 50 year (*n*=52)	10 (19.2%)		
Graft		0.647	0.917
Right (*n*=66)	17 (25.7%)		
Left (*n*=14)	3 (21.4%)		
MELD		0.837	0.806
> 25 (*n*=17)	4 (23.5%)		
< 25 (*n*=63)	16 (25.3%)		