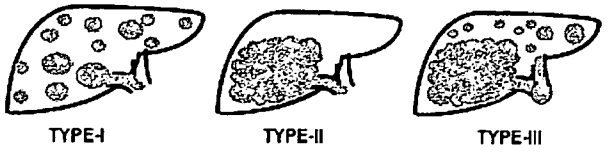

FIGURE 3. Schematic diagram of the 3 types of advanced hepatocellular carcinoma (HCC) with portal venous tumor thrombus (PVTT) in the main trunk of the portal vein according to the progression. Type I indicates PVTT with multiple nodules in both lobes; type II, PVTT with a huge mass in 1 lobe and no intrahepatic metastatic nodules in the other lobe; type III, PVTT with a huge mass in 1 lobe and multiple intrahepatic metastatic nodules in the other lobe.

study underwent palliative reduction surgery, which consisted of bisegmentectomy or trisegmentectomy with extirpation of PVTT to reduce tumor volume and to reopen the portal blood flow. IFN/5-FU combination therapy for remnant multiple hepatomas in the residual liver was carried out after surgery. With regard to these 30 patients, *none* developed any major complications, and they started the IFN/5-FU combination therapy from 3 to 5 weeks after surgery. We also demonstrated the beneficial effects of IFN/5-FU combination therapy in our patients. The efficacy of such treatment was 33.3% in our patients with highly advanced HCC. Thus, the combination treatment with IFN-α and 5-FU after hepatic palliative surgery had a marked antitumor effect with an encouraging response rate. Furthermore, the clinical response translated into survival benefits, as shown in Figures 1 and 2.

It should be noted, however, that the remaining 20 of 30 patients (66.7%) in our study did not respond to the combination therapy. Among the 20 nonresponders, there was only 1 patient with NC despite the mostly chemoresistant disease. We believe that this finding may be attributed as follows: The HCC in this series was far advanced, and HCC progression was extremely rapid and aggressive despite palliative reduction surgery. Under such conditions, almost all nonresponders died within 12 months; 12 of 20 patients (60.0%) died within 6 months. For nonresponders to this treatment, however, the survival was too short to allow the receipt of another treatment modality. Therefore, accurate prediction of chemosensitivity is desirable not only to prevent the loss of a limited chance for another possible treatment but also to avoid potentially serious side effects. However, currently, there are no suitable markers with which to distinguish between patients who are likely and patients who are unlikely to respond to this combination chemotherapy.

Several mechanisms for the anticancer effects of IFN-α, with or without 5-FU, have been proposed.[28–37]

TABLE 3
Univariate Analysis for Efficacy of Interferon-α/5-Fluorouracil Combination Therapy Based on α-Fetoprotein, Protein Induced by Vitamin K Absence, Child-Pugh Score, Cancer of the Liver Italian Program Score, and Type I Interferon Receptor 2 Expression

	No. of patients		
Characteristic	CR/PR (n = 10)	NC/PD (n = 20)	*P*
Age, y			
< 60	6	8	.9999
≥60	4	12	
Sex			
Men	10	18	.7958
Women	0	2	
Child-Pugh score			
A	7	12	.8934
B, C	3	8	
Cirrhosis			
Present	4	10	.8971
Absent	6	10	
AFP, ng/mL			
< 400	5	6	.503
≥400	5	14	
PIVKA-II, mAU/mL			
< 65	0	1	.9999
≥65	10	19	
Okuda score			
1	4	7	.7137
2-3	6	13	
CLIP score			
0-3	5	4	.2049
4-6	5	16	
IFNAR2			
Negative	0	10	.0199
Positive	10	10	

CR indicates complete response; PR, partial response; NC, no change; PD, progressive disease; AFP; α-fetoprotein, PIVKA-II; protein induced by vitamin K absence; CLIP, Cancer of the Liver Italian Program; IFNAR2, type I interferon receptor 2.

We demonstrated previously that IFN-α and 5-FU synergistically inhibited tumor cell proliferation with cell cycle arrest[38] and induced apoptosis by regulating apoptosis-related molecules.[39] We also reported that tumor necrosis factor-related apoptosis inducing ligand, its receptor pathway,[40] and Fas and the Fas-ligand pathway[41] partially contributed to the antitumor effects of IFN-α and 5-FU combination therapy. Moreover, IFN-α suppressed proliferation in all type I IFNAR2-positive HCC cell lines in vitro through mechanisms related to apoptosis or cell cycle inhibition.[42] The importance of IFNAR2 expression for the anticancer effect of IFN/5-FU was highlighted in a similar situation in our previous report.[38,39,43] These findings suggest that the antineoplastic effects of IFN-α are likely to be mediated through its high-affinity

membrane type I receptor, IFNAR2.[44] In this regard, we postulated that IFNAR2 expression in HCC tissues may be a useful predictor with which to distinguish between potential responders and nonresponders to IFN/5-FU combination therapy. On the basis of these results, we investigated the correlation between IFNAR2 expression and the effect of IFN/5-FU combination therapy using immunohistochemical analysis, and the results showed a good correlation.

Several markers for the prediction of tumor recurrence and prognosis have been identified for patients with HCC. Levy and Sherman[45] reported that the CLIP classification for HCC is easier to implement and more accurate than the Okuda classification. In addition, Koike et al.[46] suggested that the serum PIVKA-II level is the most useful clinical parameter for predicting the development of portal vein invasion. To investigate the applicability of these clinical parameters, AFP, PIVKA-II, Okuda scores, and CLIP scores were used in the current study to assess the clinical effects of IFN/5-FU combination therapy. The results indicated that expression of IFNAR2 was the only significant predictor of clinical outcome of IFN/5-FU combination therapy; and our survival analysis indicated a significant role of IFNAR2 expression on prognosis. These results suggest that the expression of IFNAR2 may be a potentially useful predictor of response to IFN/5-FU combination therapy. In our recent report using microarray analysis, several genes involved in IFN signaling transduction were identified as useful for molecular prediction of response to IFN/5-FU combination therapy.[47]

In conclusion, the current study has demonstrated the efficacy of IFN/5-FU combination therapy after surgery for patients with advanced HCC who have tumor thrombi in major branches of the portal vein. The results also indicated that the clinical response to such therapy is correlated significantly with the expression of IFNAR2 in patients with HCC.

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Case report

Complete remission of hepatocellular carcinoma with portal vein tumor thrombus and lymph node metastases by arterial infusion of 5-fluorouracil and interferon-α combination therapy following hepatic resection

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We report two cases of hepatocellular carcinoma (HCC) with portal vein tumor thrombus (PVTT) and lymph node (LN) metastases successfully treated by hepatic arterial infusion of 5-fluorouracil (5-FU) combined with systemic injection of interferon (IFN)-α following hepatic resection for the liver tumor. Complete remission was obtained. Case 1 was a 51-year-old man who had HCC in the right lobe of the liver with PVTT and multiple intrahepatic metastases. He also had abdominal and mediastinal LN metastases. Case 2 was a 53-year-old man who had diffuse-type HCC in the right lobe of the liver with PVTT and intrahepatic metastases. A chest computed tomography scan revealed lymph nodes enlarged to 1.0 cm from the mediastinum to the left supraclavicular space. Both patients underwent the hepatectomy to reduce the tumor volumes and remove the PVTT to relieve portal vein obstruction. Following the surgery, the patients underwent IFN-α/5-FU combination therapy. Three months after this combined therapy, tumor markers (both α-fetoprotein and protein induced by vitamin K absence or antagonist II) returned to the normal range and residual tumors in the liver disappeared. The patients are alive without any recurrence more than 1 year after initial treatment. IFN-α/5-FU combined therapy following hepatic resection is a promising modality for the treatment of advanced HCC with LN metastasis.

Key words: hepatocellular carcinoma, extrahepatic metastasis, lymph node metastasis, chemotherapy, interferon

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies worldwide and the fifth leading cause of cancer-related death.[1] In recent years, the development of a diagnostic modality has brought about earlier diagnosis of small HCC, and the introduction of new therapeutic modalities, such as microwave coagulation therapy and radio frequency ablation therapy, has produced various options for the treatment of small HCC.[2,3] However, the overall prognosis of HCC is still poor, since half of HCC patients have a portal vein tumor thrombus (PVTT) or intrahepatic metastasis at the time of diagnosis. Tumor progression into the major branch of the portal vein is commonly considered as the most advanced and noncurable stage,[4] and HCC with lymph node metastasis is usually considered to be a rare and far advanced stage.[5] In fact, the prognosis of HCC patients with tumor thrombus in the main portal branch (PVTT) is very poor and a standard treatment regimen for HCC with PVTT has not yet been established.[6,7] We previously reported excellent efficiency of arterial infusion of 5-fluorouracil (5-FU) and interferon (IFN)-α combination therapy for HCC with PVTT.[8,9] The eligibility criteria for this combination therapy are histologically or radiologically confirmed HCC, the presence of a tumor thrombus that has invaded at least one of the main branches of the portal vein, and the absence of hematological extrahepatic metastasis.

In this paper, we report two cases of HCC patients with PVTT and distant lymph node metastases successfully treated by 5-FU/IFN-α combined therapy following hepatic resection. This is the first report showing effective chemotherapy for patients with HCC with PVTT and extrahepatic lymph node metastasis.

Case reports

Case 1

A 51-year-old man complaining of acute upper right abdominal pain visited his neighborhood hospital, and his illness was diagnosed as chronic hepatitis due to infection with hepatitis C virus and HCC with PVTT. He was referred to our hospital for treatment of the disease. From abdominal computed tomography (CT), an early enhanced lesion (6.0cm in size) with a nonenhanced area inside was recognized in the right lobe of the liver with multiple intrahepatic metastases around the main tumor, and PVTT was developing from the anterior and posterior branch into the main portal trunk (Fig. 1A). Furthermore, the paraaortic lymph node was enlarged to 2.5cm (Fig. 1B). A chest CT scan showed the subclavicular lymph node enlarged to 2.0cm (Fig. 1C). We diagnosed the disease as huge HCC with PVTT in the right lobe of the liver and extrahepatic lymph node metastases.

Laboratory data were as follows: total bilirubin, 0.7mg/dl; aspartate aminotransferase (AST), 22U/l; alanine aminotransferase (ALT), 21U/l; prothrombin time, 64%; indocyanine green 15min retention test (ICG-R15), 16.0% (normal value, <10%), α-fetoprotein (AFP), 28ng/ml; protein induced by vitamin K absence or antagonist II (PIVKA-II), 1847mAU/l; and albumin, 3.1g/dl.

To improve the long-term outcome, we performed volume reduction surgery for the hepatic tumor and paraaortic lymph node metastasis and removed the PVTT to relieve portal vein occlusion. Histological examinations showed that the swelling lymph node and main tumor in the liver were HCC (Fig. 2). Furthermore, we investigated the immunohistochemical expression of type I interferon receptor (IFNAR2) in the primary liver tumor, PVTT, and metastatic lymph node. The immunohistochemical procedure was performed using an EnVision+ peroxidase kit (Dako, Glostrup, Denmark) as previously described.[10,11] Briefly, formalin-fixed paraffin-embedded sections were used. After deparaffinization and rehydration, the sections were treated for antigen retrieval and blocked endogenous peroxidase. Primary antibody, rabbit anti-IFNAR2 (Otsuka Pharmaceutical, Tokushima, Japan, diluted 1:60) was applied to slides and incubated overnight at 4°C. Immunostaining was performed according to the instructions supplied by the manufacturer. For evaluation of immunostaining, the bile duct epithelium expresses moderate levels of IFNAR2, and these levels of staining were used as an endogenous positive control within the

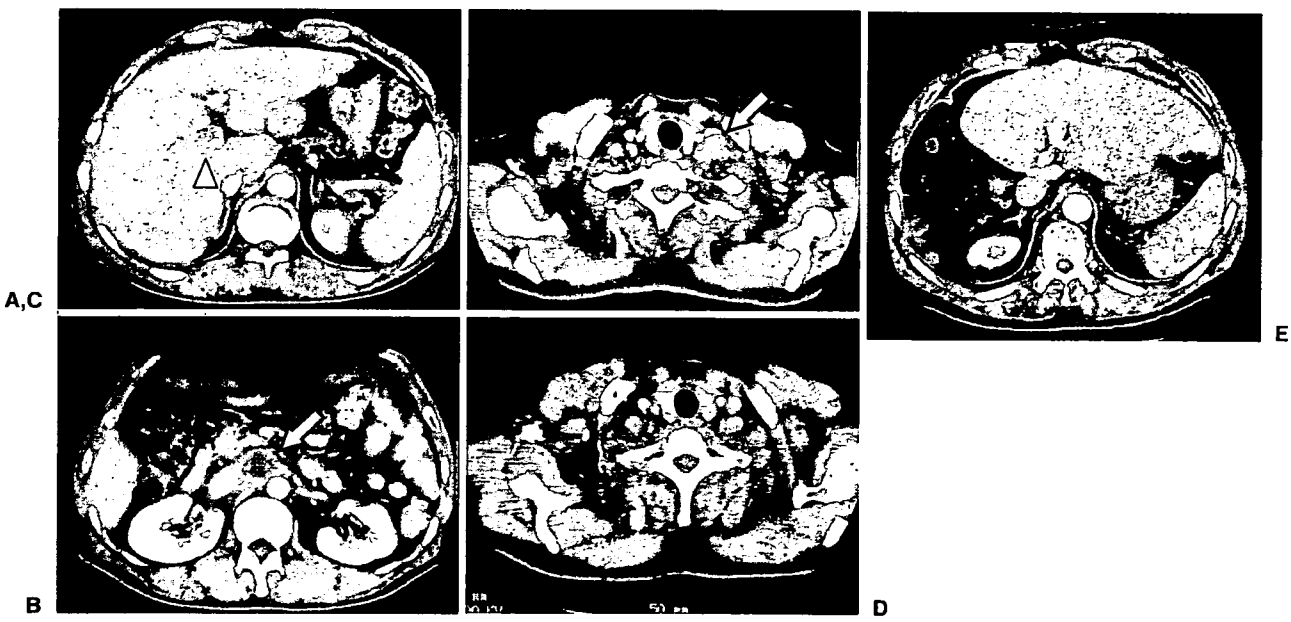

Fig. 1A–E. Computed tomography (CT) of case 1. **A** The main tumor, 6.0cm in size, is located in the right lobe of the liver, and the tumor thrombus has invaded the right main branch of the portal vein (*arrowhead*). **B** Paraaortic lymph node enlarged, 2.5cm in size (*white arrow*). **C** Chest CT scan shows an enlarged subclavicular lymph node to 2.0cm (*white arrow*). **D** After three cycles of interferon-α/5-fluorouracil combination therapy, the subclavicular lymph node had vanished. **E** There was no residual tumor or recurrence in the remnant liver 56 months after the first treatment

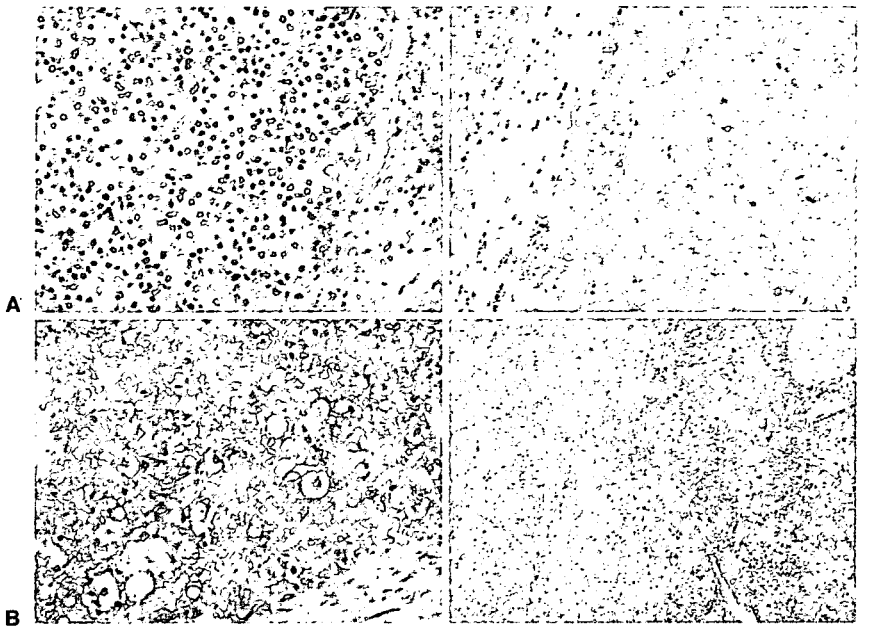

Fig. 2. **A** Microscopic finding of a metastatic lymph node of case 1 showed moderately differentiated hepatocellular carcinoma (hematoxylin and eosin, ×200). **B, C** Immunohistochemical staining for type I interferon receptor (IFNAR2) in the metastatic lymph node of case 1 (**B**) and in the main tumor of case 2 (**C**) showed strong expression of IFNAR2 (×200). **D** Microscopic finding of the main tumor in the liver showed poorly differentiated hepatocellular carcinoma (hematoxylin and eosin, ×100)

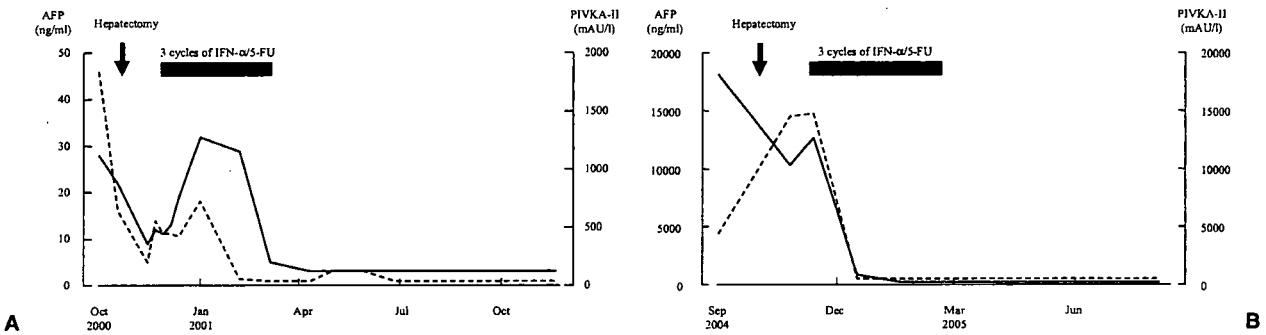

Fig. 3A,B. Clinical courses of cases 1 (**A**) and 2 (**B**). Serum α fetoprotein (*AFP*) and protein induced by vitamin K absence or antagonist II (*PIVKA-II*) are indicated by the *solid line* and *dashed line*, respectively. IFN-α/5-FU, interferon-α/5-fluorouracil combination therapy

sample, as described previously. The expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node were positive (Fig. 2). Following the surgery, the patient was treated with subcutaneous administration of IFN-α (OIF; Otsuka Pharmaceutical, Tokyo, Japan) and continuous hepatic arterial infusion of 5-FU (Kyowa Hakko, Tokyo, Japan). The regimen was as follows: IFN-α (5×10^6 U) was administered on days 1, 3, and 5 of every week for 4 weeks and continuous hepatic arterial infusion chemotherapy (5-FU, 300 mg/m^2) was performed every 2 weeks for 4 weeks via a catheter connected to a subcutaneously implanted drug delivery system. Three months after the initial chemotherapy, tumor markers (both AFP and PIVKA-II) returned to normal range (Fig. 3A) and a CT scan showed that the subclavicular lymph node had disappeared (Fig. 1D). The patient still lives without any residual tumor or recurrence in either the liver or the extrahepatic sites, 56 months after the first treatment (Fig. 1E).

Case 2

A 53-year-old man had been regularly followed up for chronic hepatitis C at a local hospital. Regular follow-up abdominal CT revealed a single mass, 1.0 cm in diameter, in the right posterior superior segment (segment 7) of the liver in July 2003. He was diagnosed as having solitary HCC, and percutaneous ethanol injection therapy (PEIT) and transcatheter arterial embolization

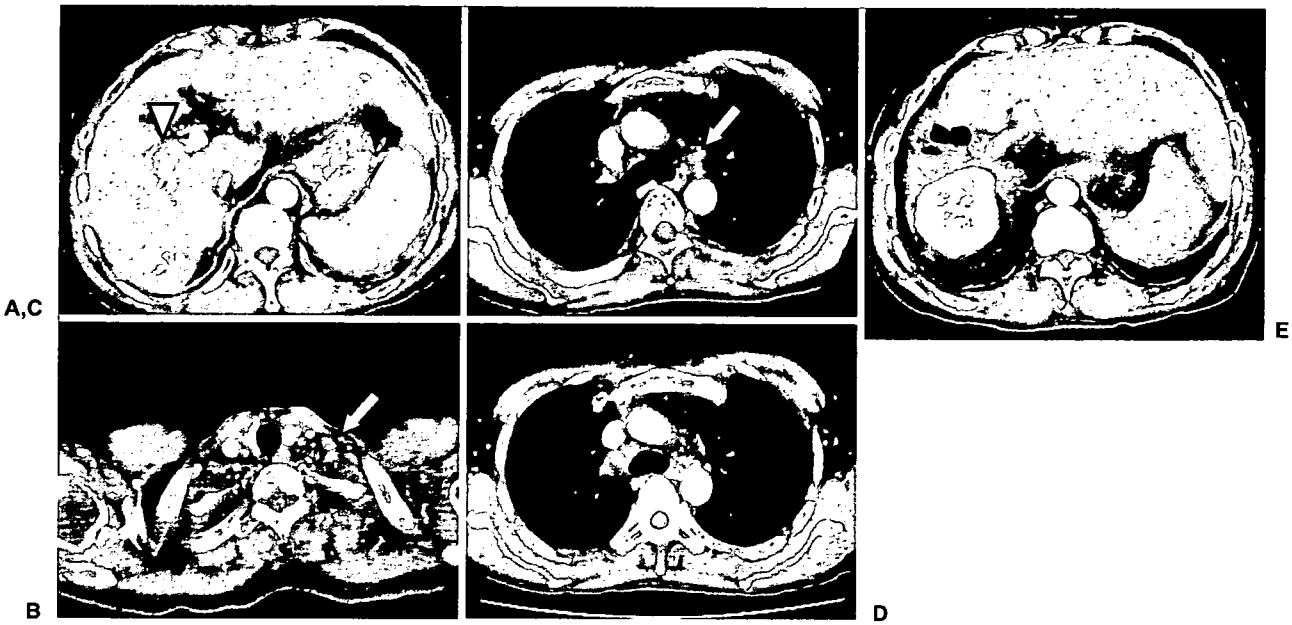

Fig. 4A–E. CT of case 2. **A** The main tumor is an early enhanced area occupying the whole right lobe of the liver, and the tumor thrombus has invaded the right main branch of the portal vein (*arrowhead*). **B** Cervical CT scan showing the enlarged supraclavicular lymph node (*white arrow*). **C** Chest CT scan shows the enlarged mediastinum lymph nodes (*white arrow*). **D** After three cycles of interferon-α/5-fluorouracil combination therapy, the mediastinum lymph nodes had diminished in size. **E** Residual tumors in the left lobe of the liver disappeared and there was no recurrence in the liver 12 months after hepatectomy

(TAE) was performed. Thirteen months after the treatment, CT scan and angiography showed a huge mass with PVTT extending to the portal trunk in the right lobe of the liver and four intrahepatic metastases in the left lobe of the liver. After receiving TAE for the intrahepatic metastases in the left lobe of the liver from the left hepatic artery and transcatheter arterial infusion (TAI) therapy with cisplatin (30 mg), mitomycin (6 mg), and daunorubicin (20 mg) from the right hepatic artery, he was referred to our hospital for further treatment. On admission, abdominal CT 1 month after TAE and TAI showed a dense accumulation of iodized oil in the intrahepatic metastases in the left lobe and an early enhanced area in the whole right lobe of the liver (Fig. 4A). The PVTT had developed from both the right anterior and posterior branches into the main trunk of the portal vein. Chest CT scan revealed enlarged lymph nodes, 1.0 cm in size, from the mediastinum to the left supraclavicular space (Fig. 4B, C).

Laboratory data were as follows: total bilirubin, 1.0 mg/dl; AST, 42 U/l; ALT, 28 U/l; prothrombin time, 59%; ICG-R15, 20%; AFP, 17920 ng/ml; PIVKA-II, 3873 mAU/ml; and albumin, 3.5 g/dl.

We performed a right lobectomy of the liver to reduce the tumor volumes and removed the PVTT to relieve portal vein obstruction. Following the surgery, the patient was treated with subcutaneous administration of IFN-α and continuous hepatic arterial infusion chemotherapy of 5-FU. The patient underwent open biopsy of the enlarged supraclavicular lymph node 1 month after the surgery, and we histologically confirmed that the swelling lymph node was metastasis from HCC. Immunohistochemical examination showed positive expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node (Fig. 2). Three months after the initial chemotherapy, tumor markers (both AFP and PIVKA-II) returned to normal range (Fig. 3B), and a CT scan showed that residual tumors in the left lobe of the liver had disappeared and that the enlarged lymph nodes in the mediastinum had diminished in size (Fig. 4D). Twelve months after the surgery, the patient is still alive without tumor recurrence (Fig. 4E).

Discussion

The incidence of lymph node (LN) metastasis in HCC is generally rarer than that in other cancers. The incidence of LN metastasis from HCC is reported to be 25%–42% at autopsy.[12–14] In clinical series, the incidence rate of LN metastasis from HCC is lower than at autopsy, because LN dissection is not routinely performed during hepatectomy for patients with HCC, and LN

metastases occur in patients with far advanced and unresectable HCC. The prognosis of HCC with LN metastases is extremely poor. The cause of death with LN metastases from HCC is usually liver failure due to the progression of liver tumors.[15] Uenishi et al.[5] reported that six HCC patients with LN metastasis at primary surgery died within 424 days owing to tumor progression or liver failure. Furthermore, all patients had intrahepatic metastases, and five had PVTT.

Our two patients had PVTT. The prognosis of HCC patients with PVTT is also extremely poor: median survival time is only 2.7 months.[16] Furthermore, the presence of extrahepatic metastasis is one of the most important negative prognosis factors. According to previous reports, there is no survival benefit of systemic chemotherapy for patients with advanced HCC, and the median survival time is less than 4 months.[17] Previously, we and others reported that chemotherapy with 5-FU combined with systemic administration of INF-α is effective against HCC.[8,11,18] Our recent report showed that arterial infusion chemotherapy combined with systemic administration of IFN-α is very effective for unresectable HCC with PVTT, and the median survival time of the patients who received this combined therapy was 10.2 months.[11] Furthermore, we recently reported a clinical trial in which hepatic resection followed by IFN-α and 5-FU improved the prognosis of HCC patients with PVTT compared with surgery alone.[19] Therefore, in our two cases, we determined that the hepatic arterial infusion of 5-FU combined with systemic administration of IFN-α should be administered as adjuvant treatment after hepatectomy.

Yatsuhashi et al.[20] showed that efficacy of IFN therapy is related to the expression of IFNAR2. In transfected cancer cell lines showing enhanced expression of IFNAR2, the antiproliferative effects of IFN were markedly increased.[21] In a clinical trial of IFN-α/5-FU combined therapy for 55 patients with unresectable HCC with PVTT, we revealed that the efficacy of this combined therapy is significantly related to the expression of IFNAR2.[11] Therefore, we investigated the immunohistochemical expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node of both these cases. In both cases, the expression of IFNAR2 in the primary liver tumor, PVTT, and metastatic lymph node were positive. These results suggest that IFN-α/5-FU combined therapy is effective not only against the primary liver tumor but also against LN metastasis in which IFNAR2 is expressed.

Generally, lymph node metastasis is considered to be spread hematogenously to distant organs, similar to metastasis to other organs.[22] In this regard, IFN/5-FU therapy may be effective against systemic circulating hematogenously spread metastasis. This suggests that IFN/5-FU might be a useful adjuvant therapy not only following hepatic resection in HCC with good liver function, but also following hepatic transplantation in HCC with poor liver function.

In conclusion, IFN-α/5-FU combined therapy following hepatic surgery may be a promising modality for advanced HCC with PVTT, even with lymph node metastasis.

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Original Article

New chemotherapy for patients with advanced hepatocellular carcinoma: Pilot study of β-interferon and doxorubicin one-shot intra-arterial chemotherapy

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Background: Patients with advanced hepatocellular carcinoma (HCC) need an effective treatment modality because of the poor prognosis of the disease. From an *in vitro* study, β-interferon (IFN-β) has been reported to enhance the antiproliferative effects of doxorubicin on HCC cell lines. In the present study, we investigated the therapeutic effects of combined IFN-β and doxorubicin intra-arterial injection therapy on patients with advanced HCC.

Methods: IFN-β (3 MIU) and doxorubicin (10 mg/bodyweight) were given by one-shot intra-arterial injection through an arterial port to patients with advanced HCC. One treatment course consisted of three intra-arterial injections per week for 4 weeks. Three courses were conducted and evaluation was done monthly.

Results: Eleven patients with advanced HCC were treated with combined IFN-β and doxorubicin. One patient entered complete remission (CR), seven patients were evaluated as having stable disease (SD) and three as having progressive disease (PD). The mean overall survival was 10 months. The mean survival for CR and SD patients was 15 months, and that for PD patients was 6 months ($P = 0.0464$, log–rank test). Decrease of serum total bilirubin was observed for all patients.

Conclusion: Combined IFN-β and doxorubicin intra-arterial therapy offers an effective chemotherapy option for patients with advanced HCC by improving liver function and having tolerable side-effects.

Key words: advanced hepatocellular carcinoma, β-interferon, doxorubicin, intra-arterial injection

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is principally associated with hepatitis B virus (HBV) or hepatitis C virus (HCV), and its incidence is especially high in Asia and Africa.[1] Recently, its incidence has been increasing in Europe and America.[2,3] There are various options for treatment of HCC, including radiofrequency ablation (RFA), percutaneous ethanol injection (PEI), transcatheter arterial embolization (TAE) using interventional radiology (IVR), surgical resection, and liver transplantation.[4] However, the prognosis is poor for patients with advanced hepatic carcinomas, which develop in multiple segments in the liver and/or are accompanied by portal vein tumor thrombus, because no efficacious treatment modality has yet been developed.[5] Recently, for patients with advanced HCC without metastatic foci whose performance status (PS) is good, approximately 50% effectiveness has been reported for combined α-interferon (IFN-α) and 5-fluoruracil (5-FU) arterial injection therapy.[6,7] For patients with poor liver function who cannot accept IFN-α and 5-FU combination therapy, a new chemotherapy regimen is needed. Thus, we designed a protocol that minimizes hepatic toxicity and also enables one-shot arterial injection for patients with advanced HCC, who are not candidates for operation, liver

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transplantation, or local treatment such as IVR, PEIT or RFA due to the number of tumors, portal vein thrombosis, or liver dysfunction (BCLC staging system B or C).[8]

β-interferon (IFN-β) is usually given by injection into the bloodstream and has fewer side-effects than IFN-α.[9] Recently, an *in vitro* study has shown that IFN-β could suppress the proliferation of HCC more strongly than IFN-α both alone and in combination with anticancer agents.[10] In particular, the antitumor agent doxorubicin showed synergism with IFN-β in the antiproliferation effect against HCC using HCC cell lines.[11] As myocardial damage and hepatic toxicity are the main side-effects of doxorubicin,[12,13] a small-volume one-shot arterial injection was selected for giving IFN-β. This led us to design a new chemotherapy regimen of combined IFN-β and doxorubicin intra-arterial injection therapy. The present study was conducted to determine whether this combined chemotherapy could be used for outpatient treatment after a short hospital stay in order to maintain the patient's quality of life (QOL) with fewer side-effects.

METHODS

Patient enrollment

PATIENTS WITH CIRRHOSIS and advanced HCC who were enrolled in this study were not eligible for surgical resection, liver transplantation or local treatment such as IVR, PEI or RFA because of diffuse or multiple tumors in both lobes with or without portal vein tumor thrombus and/or impaired liver function due to cirrhosis. To realize chemotherapy on an outpatient basis, patients with PS 0 or 1 were selected. Informed consent was obtained after explaining the purpose of the study and possible side-effects. Clinical tumor stages of patients with HCC were evaluated by abdominal contrast enhanced computed tomographic (CT) scans, magnetic resonance images (MRI) or angiography. Other criteria were a neutrophil count $\geq 1000/mm^3$, platelet count $\geq 40\,000/mm^3$, serum level of creatinine ≤ 1.4 mg/dL, total bilirubin of ≤ 3.5 mg/dL, and no abnormalities of cardiac function by ultrasound and electrocardiography. The exclusion criteria included intractable pleural effusion or ascites, severe infectious disease, severe myocardial damage, severe impairment of intelligence, encephalosis, metastasis to the central nervous system, hemorrhage from varicose veins within 1 month prior to enrollment, and pregnancy.

Therapeutic design

All of the enrolled patients had a catheter placed by gastroduodenal artery (GDA) coil or other method and a port implanted subcutaneously. One course of chemotherapy consisted of one-shot intra-arterial injection of IFN-β (3 MIU) and doxorubicin (10 mg/bodyweight) through the port, three times per week for 4 weeks. Three courses were conducted, when possible, and monthly evaluation of chemotherapy effects on HCC was based on serum tumor markers and CT scans.

Evaluation of therapeutic effects

The antitumor effect was evaluated by tumor volumes using contrast enhanced CT scans every 4 weeks from the start of combined IFN-β and doxorubicin intra-arterial injection therapy. The antitumor effect and toxicity were evaluated according to National Cancer Institute Common Toxicity Criteria (NCI-CTC)[14] and Response Evaluation Criteria in Solid Tumors (RECIST)[15] guidelines. Peripheral blood cells, biochemical tests, serum levels of α-fetoprotein (AFP) and/or PIVKA-II were examined every 4 weeks. The overall survival was calculated from the first treatment until death or the final day of follow up. The primary end-point of the current study was the development of toxicity and overall survival.

The criteria of complete response (CR), stable disease (SD) and progressive disease (PD) were as follows: CR, complete disappearance of tumors and no evidence of new lesions; SD, < 50% reduction or < 25% increase of tumor volume and no evidence of new lesions; PD, ≥25% increase of tumor volume, evidence of new lesions, or rise in tumor markers.

Statistics

The overall survival time from the start of the chemotherapy was analyzed by the Kaplan–Meier method and differences in survival were evaluated by log-rank tests.

RESULTS

Patient characteristics

ELEVEN PATIENTS WERE enrolled at Osaka University Hospital between November 2003 and August 2005. HCC was diagnosed by contrast-enhanced CT scan or MRI. Angiography and pathological diagnosis were not done. The serum levels of AFP and PIVKA-II were elevated. The pretreatment characteristics of enrolled patients are shown in Table 1.

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Table 1 Pretreatment characteristics of patients with advanced hepatocellular carcinoma

No.	Age (years)	Sex	Etiology	Child-Pugh grade	Portal venous thrombosis (Vp)	Previous treatment
1	56	M	HBV/HCV	B	+	TAE
2	78	M	HCV	A	+	TAE, RFA
3	73	M	HBV	A	-	Operation, TAE
4	58	M	HCV	B	-	TAE
5	71	M	HCV	B	-	TAE
6	49	M	HCV	C	+	TAE, RFA
7	69	M	Non B/non C	B	+	None
8	63	M	HBV	A	+	TAE, RFA
9	62	F	HCV	B	-	TAE
10	61	M	HCV	A	+	TAE, RFA
11	56	M	HCV	A	-	None

HBV, hepatitis B virus; HCV, hepatitis C virus; RFA, radiofrequency ablation; TAE, transcatheter arterial embolization.

All patients were enrolled after being diagnosed as having liver cirrhosis by biochemical tests and/or radiological findings. Histological confirmation of liver cirrhosis was not done. The liver function of patients with cirrhosis was classified according to Child-Pugh grading criteria. Pretreatment tumor stages of patients with advanced HCC were classified according to the American Joint Committee on Cancer (AJCC) Tumor-Lymph Node Metastasis (TNM) classification system,[16] and according to the Cancer of the Liver Italian Program (CLIP) score[17] (Table 2). Seven patients had HCV infection, two had HBV, one had both HBV and HCV. One patient suffered from cirrhosis with neither HBV nor HCV infection.

Tolerability and side-effects

Eleven patients were started with intra-arterial administration of 3 MIU IFN-β and 10 mg doxorubicin. The median period of combined chemotherapy was 11 weeks (range 8–12 weeks). The dose of doxorubicin was reduced from 10 mg/bodyweight to 5 mg/bodyweight for two patients (nos. 2 and 6) because of grade 3 and 4 neutropenia. A 78-year-old man (no. 2) developed grade 4 neutropenia after the first course, and doxorubicin was reduced to 5 mg/bodyweight and granulocyte-colony stimulating factor (G-CSF) was given, and then grade 4 stomatitis appeared after two courses leading to discontinuation of the chemo-

Table 2 Therapeutic effect according to RECIST on patients and tumor stages of HCC patients according to the CLIP score and TNM classification system

No.	T-Bil (mg/mL)	AFP (ng/mL)	PIVKA II (mAU/mL)	CLIP score	TNM	Duration of therapy	Therapeutic effect	Prognosis
1	1.9	<5.3	<40	4	III	3 cycles	SD	15 M Dead
2	1.7	2 145	<40	4	IVA	2 cycles	PD	6 M Dead
3	0.6	24	148	1	III	3 cycles	SD	8 M Dead
4	3.3	24	140	2	III	3 cycles	SD	35 M Alive
5	1.6	25	462	3	III	2 cycles	SD	6 M Dead
6	2.1	10 400	32 852	6	IVB	3 cycles	SD	6 M Dead
7	1.3	226 820	12 317	5	IVA	3 cycles	PD	5 M Dead
8	2.4	582	63	3	IVA	3 cycles	CR	20 M Alive
9	2.9	41	1 397	2	IVA	2 cycles	SD	12 M Dead
10	0.7	255	1 341	3	III	3 cycles	PD	10 M Dead
11	2.4	309	13 900	1	III	3 cycles	SD	25 M Alive

AFP, α-fetoprotein; CLIP score, Cancer of the Liver Italian Program score; CR, complete remission; PD, progressive disease; RECIST, Response Evaluation Criteria in Solid Tumors; SD, stable disease; T-Bil, total bilirubin; TNM, Tumor-lymph Node Metastasis classification system.

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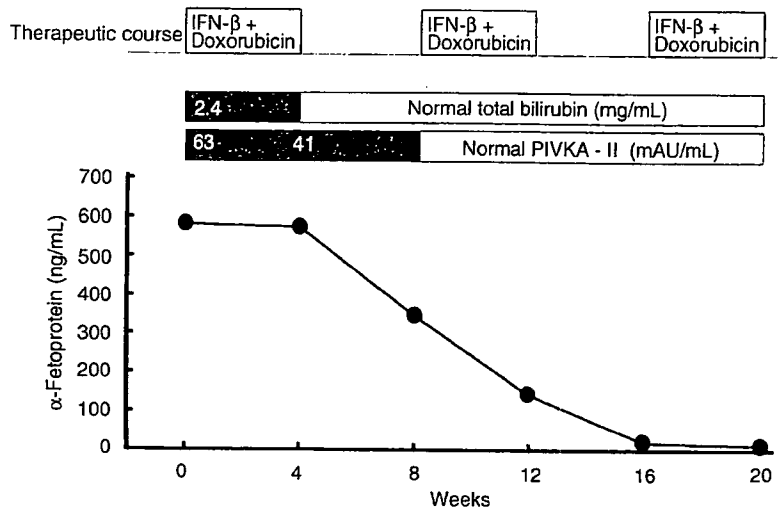

Figure 1 Time course of tumor markers in a complete remission case. A 63-year-old man with diffused type advanced hepatocellular carcinoma (HCC) (no. 8) was treated with three courses of combined β-interferon (IFN-β) and doxorubicin intra-arterial injection therapy without severe side-effects. Serum levels of PIVKA-II decreased after the first course of combined chemotherapy, entered the normal range during the second course and remained in the normal range after three courses. The serum level of α-fetoprotein decreased after the second course and entered the normal range 1 month after three courses of combined therapy. No HCC lesions were detected in the patient's liver by contrast enhanced CT scans and MRI after three courses of combined chemotherapy and 6 months later.

therapy. A 71-year-old man (no. 5) and a 62-year-old woman (no. 9) with Child–Pugh grade B complained of severe fatigue after two courses, and the chemotherapy was stopped. They had been treated by TAE for the tumors more than five times previously. Previous treatments, especially transarterial chemoembolization (TACE) may have affected the severity of the toxicity of the present combined chemotherapy regimen, although other factors such as age and Child–Pugh grade can be considered as having affected the development of intolerable side-effects. Discontinuation of drug therapy led to quick recovery from the adverse reactions. Of the eight remaining patients, three dropped out of the study and five completed three courses of treatment.

Therapeutic effects of combined intra-arterial IFN-β and doxorubicin injection therapy

All patients had advanced HCC, seven with and four without portal thrombus. All HCC were evaluated for volume changes by contrast-enhanced CT scans after 8 or 12 weeks. A 63-year-old man (no. 8) with HBV infection showed significant reduction of AFP and PIVKA-II into the normal range. Diffuse HCC disappeared after three courses of combined IFN-β and doxorubicin intra-arterial injection therapy, being confirmed by contrast-enhanced CT scan and MRI. Thus, we concluded that patient no. 8 had attained CR (Fig. 1).

All patients showed a high serum level of AFP and/or PIVKA-II before treatment (Table 2). The serum levels of AFP and/or PIVKA-II decreased after one course of combined chemotherapy in all patients. However, the CT scans demonstrated no significant volume reduction of HCC in seven patients, and tumor enlargement in three. Seven patients were classified as SD and three as PD from contrast-enhanced CT scans (Table 1).

Overall survival

All of the patients were observed from November 2003 to October 2006. The estimated duration of overall median survival was 10 months (Fig. 2a). The mean survival time was 15 months for CR and SD patients, which is significantly longer than 6 months for PD patients ($P = 0.0464$, log-rank test) (Fig. 2b). The mean survival time of only SD patients (12 months) was not significantly longer than that for PD patients ($P = 0.0786$, log-rank test). The one-year survival rate for CR and SD patients was 62.5% (5/8) and that for PD was 0% (0/3). The progression-free survival time for CR or SD was longer than that for PD ($P = 0.0004$, log-rank test)

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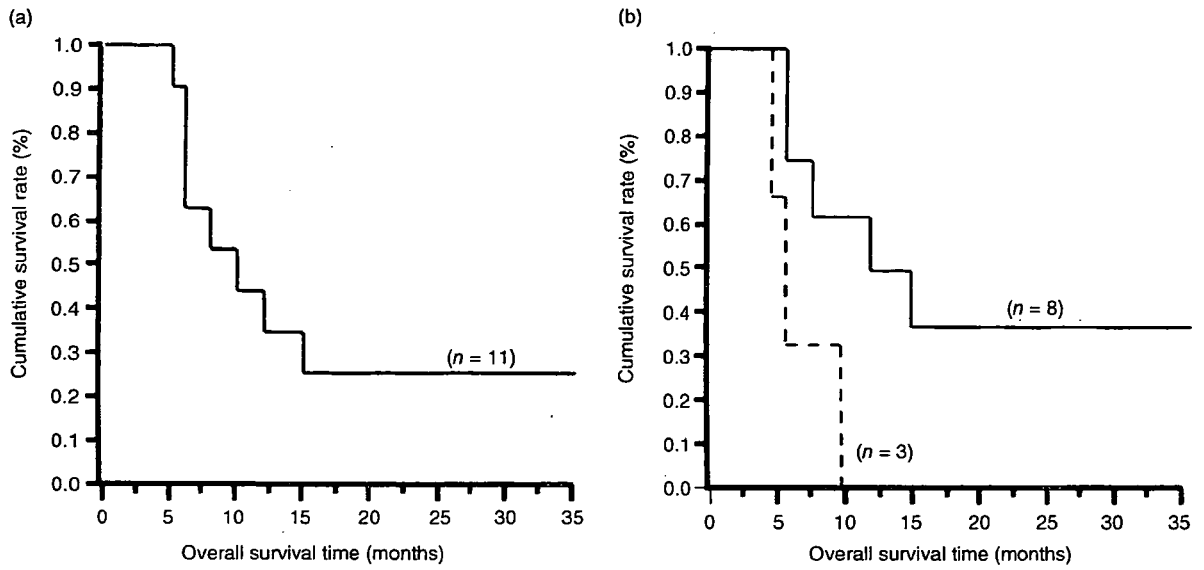

Figure 2 Overall survival periods of patients with advanced hepatocellular carcinoma who received combined β-interferon (IFN-β) and doxorubicin intra-arterial injection therapy. (a) Overall survival periods of 11 patients who received combined IFN-β and doxorubicin intra-arterial injection therapy. The mean survival period was 10 months. (b) Overall survival periods of seven patients with stable disease (SD) and three with progressive disease (PD) after combined IFN-β and doxorubicin intra-arterial injection therapy. The mean survival period was 15 months for SD patients and 6 months for PD patients. (——), CR-SD; (- - -), PD. CR, complete response. ($P = 0.0464$, log-rank test).

(Fig. 3). Eight patients died of liver failure, including five SD and three PD patients. A 73-year-old man (no. 3) died of sepsis that developed from catheter problems, after completion of three cycles of treatment. Three patients are alive, including one CR patient (25 months) and two SD patients (35 and 20 months). The QOL of PD patients was maintained until the end of the treatment. The Eastern Cooperative Oncology Group (ECOG) performance status at the end of the treatment had not deteriorated.

Total bilirubin of the HCC patients who had received IFN-β and doxorubicin intra-arterial combination therapy decreased significantly after one cycle ($P = 0.0344$) and two cycles ($P = 0.0051$) of treatment (Fig. 4). In all patients, anorexia and lassitude were alleviated, offering remarkable benefits for advanced HCC patients.

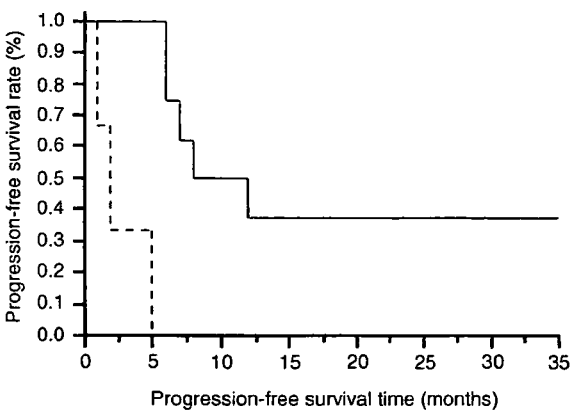

Figure 3 Progression-free survival times of patients with advanced hepatocellular carcinoma according to responses to β-interferon and doxorubicin combination therapy. One-year survival rate for CR or SD patients was 62.5% (5/8) and that for PD was 0% (0/3). The progression-free survival time for CR or SD was longer than that for PD. (——), CR-SD; (- - -), PD. CR, complete response. ($P = 0.0004$, log-rank test).

DISCUSSION

HEPATOCELLULAR CARCINOMAS RECEIVE nourishment from the hepatic artery, not the portal

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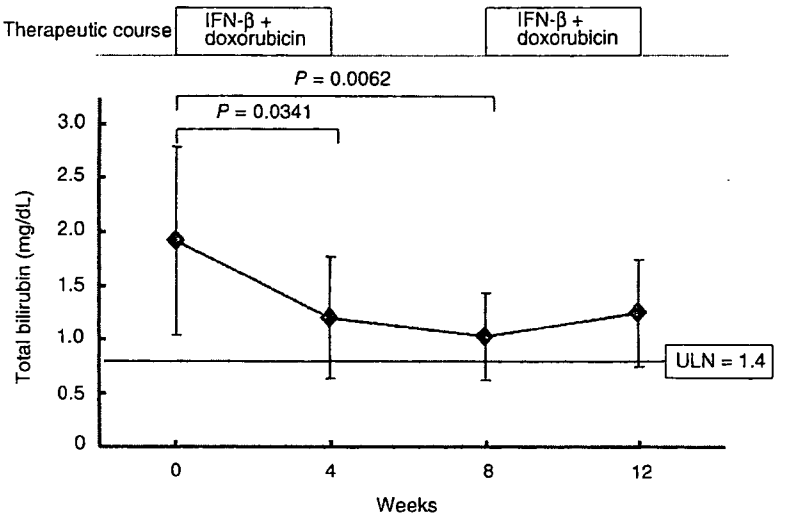

Figure 4 Serum bilirubin ameliorated during combined β-interferon (IFN-β) and doxorubicin intra-arterial injection therapy. Serum levels of total bilirubin decreased significantly and entered the normal range after the first course of combined chemotherapy, and remained in the normal range during the further courses. Values are averages ± SD. Upper limit of normal (ULN) serum values of total bilirubin, 1.4 mg/dL.

flow. Thus, a therapeutic effect should be attainable by giving antitumor agents via the hepatic artery. By direct delivery into the hepatic artery, the concentrations of anticancer agents in the liver increase to 10-fold or more than those by administration via the peripheral veins.[18] By direct injection of anticancer drugs into blood vessels draining to local areas, higher therapeutic effects can be expected when higher ratios of drug concentration appear in the internal organs on their first pass (first-pass effect).[19] When doxorubicin is infused from the hepatic artery, the first-pass effect in the liver is considered to be approximately 60% in rabbits. As the antitumor effects are dose dependent, anthracyclines, including doxorubicin, should be suitable for intra-arterial chemotherapy by single bolus injection.[20] Doxorubicin is metabolized in the liver by hepatic cytochrome P450 and is excreted in bile and urine.[21] On being metabolized by the typical P450 CYP3A4, 40% or more of doxorubicin is ultimately excreted via the bile. Its metabolism and excretion are delayed in patients with hepatic dysfunction such as cirrhosis or with obstructive jaundice, in whom the side-effects of anthracyclines tend to develop easily. In the present study, myelosuppression was observed in two patients (nos. 2 and 6), and in one case G-CSF had to be used. We have examined the concentration of doxorubicin of 10 patients including these patients. The blood concentration of doxorubicin was measured by high-performance liquid chromatography using patients' serum. In two patients with myelosuppression, the blood concentrations of doxorubicin exceeded 10 ng/mL at 60 min after the start of administration. In these patients, no significant hepatic damages were observed. Another eight patients without significant myelosuppression, whose blood concentration of doxorubicin could be measured, showed lower blood concentration than 10 ng/mL. These findings suggested that patients, in whom the blood doxorubicin concentration is 10 ng/mL or more at 60 min after the start of administration, seem to be susceptible to the side-effects, especially hematological toxicity. In general, the serum concentration of doxorubicin at 60 min after its administration is less than 10 ng/mL in normal subjects. But it could be well considered that the serum concentration of doxorubicin at 60 min after its administration to the patients with liver dysfuction is more than 10 ng/mL due to the delayed metabolism and excretion of doxorubicin. The monitoring of serum concentration of doxorubicin seems to be important in patients with liver cirrhosis.

IFN-β and doxorubicin intra-arterial combination therapy significantly reduced total bilirubin, but did not improve other liver function tests such as prothrombin time and albumin, This seems to be the most distinct hallmark of this therapy. In the present study, no patients had tumor thrombus in the bile duct, However, in the cases of advanced HCC, tumors may compress the small bile duct. After the treatment of combination therapy, compression of the small bile duct by tumors may be relieved because of the reduction of tumor size. However, giving IFN to bile duct-ligated rats has been reported to result in significant preservation of histology, inhibition of collagen accumulation and partial

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improvement of serum markers of cholestasis.[21] Thus, IFN used with doxorubicin may bring about the partial improvement of cholestasis in patients with advanced HCC. However, the mechanism of reduction of serum bilirubin by this combination chemotherapy remains to be clarified. Marked improvement of total bilirubin by IFN-β and doxorubicin therapy in HCC patients might offer clinical proof of the novel characteristics of interferon.

Yang *et al.* reported the efficacy of gemcitabine and doxorubicin for patients with advanced HCC, with median survival of 4.6 months for all patients and median progression-free survival of 2.5 months.[22] Obi *et al.* reported the efficacy of combination therapy of systemic IFN-α and intra-arterial 5-FU for HCC patients with portal vein invasion, with the survival rate at 12 months being 34% and median survival time of 6.9 months.[23] The 1-year survival rate for CR or SD patients was 62.5% and that for all patients, including PD patients, was 45%, and the mean survival time for all patients was 10 months in the present study, although the number of the patients was small. The present findings suggested that IFN-β is more effective than gemcitabine or IFN-α for advanced HCC. This might explain the effectiveness of IFN-β injected into the tumor site in the liver directly through the catheter. To confirm the superior effects of intra-arterial IFN-β administration, further studies with more patients and longer treatment periods should be done.

All patients enrolled in the present study had extensively advanced HCC, with five cases including portal tumor thrombus Vp3. Patients with Child–Pugh grades A and B are also eligible for this combined chemotherapy regimen, but the dose and the interval of administration should be considered for patients with ascites or a serum level of total bilirubin at 3.0 mg/dL or more, such as Child–Pugh grade C.

Small amounts of IFN-β and doxorubicin do not tend to cause severe side-effects. Under the new enrollment criteria, HCC patients need only 2 or 3 days of hospital stay for port implantation, and outpatient therapy can be started immediately. Moreover, this one-shot intra-arterial injection therapy can be conducted within a short time to minimize restriction of the patient. Based on these findings, one-shot intra-arterial combination chemotherapy of IFN-β and doxorubin could be recommended for outpatient therapy of patients with advanced HCC.

In conclusion, for patients with progressive hepatocellular carcinoma, this preliminary study shows that combined IFN-β and doxorubicin intra-arterial chemotherapy has the potential of prolonging survival time while maintaining QOL in an outpatient clinic. This combination chemotherapy, with tolerable side-effects, has the potential of serving as an optimal treatment option for advanced HCC, by improving liver function and maintaining the QOL for outpatients.

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Doxorubicin/IFN-β併用化学療法と肝切除術により長期生存し得た右心房内腫瘍栓を伴う進行肝細胞癌の1例

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A Case of Hepatocellular Carcinoma with Right Atrium Tumor Thrombus Treated with Combined Doxorubicin and Interferon-β/Intra-Arterial Injection Chemotherapy and Hepatectomy: Masahiro Murakami[*1], Hiroaki Nagano[*1], Takehiro Noda[*1], Hiroshi Wada[*1], Shogo Kobayashi[*1], Shigeru Marubashi[*1], Atsushi Miyamoto[*1], Yutaka Takeda[*1], Keizo Dono[*1], Koji Umeshita[*2] and Morito Monden[*1] ([*1]*Dept. of Surgery and* [*2]*Dept. of Health Science, Graduate School of Medicine, Osaka University*)

Summary

A-58-year-old male was admitted to Osaka University Hospital for advanced hepatocellular carcinoma in July 2005. The main tumor was located in the posterior segment and hepatic vein tumor thrombus extended to the right cardiac atrium. He felt of pressure in his chest and a serum total bilirubin level was beyond normal range because of the tumor progress. We started a doxorubicin and interferon-β combined chemotherapy. Although anti-tumor effect was NC, his symptom rather improved and a serum total bilirubin level went into the normal range. Consequently, we performed an extended posterior segmentectomy and tumor thrombectomy of IVC and right cardiac atrium. The patient survived for 13 months after the initial treatment, but he died of distant metastasis. It was suggested that the doxorubicin and interferon-β combined chemotherapy might be the promising modality for advanced hepatocellular carcinoma as one of the multimodel treatment. Key words: Hepatocellular carcinoma, Interferon-β, Doxorubicin

要旨 症例は58歳，男性。B型肝炎，多量飲酒歴あり。2005年4月疲労感などを主訴に近医を受診し，精査にて進行肝細胞癌と診断され当院へ紹介。7月精査加療目的で入院した。画像上，肝後区域の主腫瘍と肝部下大静脈から右心房内に至る腫瘍栓を認めた。入院時より胸部圧迫感や下腿浮腫などが出現，血清総ビリルビン値（T-Bil）は2.2 mg/dLと上昇し，腫瘍進展による肝不全徴候を認めた。doxorubicin/IFN-β併用化学療法の施行により，画像上の抗腫瘍効果はNCであったものの，症状の改善とT-Bilの正常化を認めたことより，10月肝拡大後区域切除，右心房内・下大静脈内腫瘍栓摘出術を施行した。術後経過は特に問題なく退院し，最終的に遠隔転移により癌死したが，初回治療より13か月の長期生存を得た。以上よりdoxorubicin/IFN-β併用化学療法は，進行肝細胞癌に対して集学的治療の有用な選択肢の一つとなり得ると思われた。

緒 言

今回われわれは肝部下大静脈をほぼ充満し，右心房内に至る広範な腫瘍栓を伴う進行肝細胞癌で腫瘍進展に伴う肝不全徴候の出現した症例に対して，doxorubicin/IFN-β併用化学療法を施行後に根治肝切除術を施行し，長期生存を得た症例を経験したので報告する。

I. 症 例

患者: 58歳，男性。HBs抗原陽性。

既往歴: 20年前より高血圧で内服中。

飲酒歴: 日本酒3合/日×38年と多量飲酒。

現病歴: 2005年4月疲労感および咳嗽を主訴に近医を受診し，心房細動を指摘。その時の腹部CT検査で進行肝細胞癌と診断され，7月精査加療目的にて入院した。入院時，胸部圧迫感や下腿浮腫などの右心房内腫瘍栓によると思われる症状が出現していた。

入院時血液検査: PT値76%と軽度低下，T-Bil 2.2 mg/dLと上昇し，腫瘍進展による肝不全徴候を認めた。腫瘍マーカーはAFP 4,014 ng/mLとPIVKA-II 56

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表 1 入院時血液検査所見

WBC	5,100/μL	APTT	30 sec
RBC	418×10⁴/μL	PT	76%
Hb	13.8 g/dL	HPT	76%
Hct	40.6%	ICG R_{15}	20%
Plt	14.6×10⁴/μL	HBs-Ag	(+)
TP	7.5 g/dL	HBs-Ab	(−)
Alb	3.8 g/dL	HBe-Ag	(−)
T-Bil	2.2 mg/dL	HBe-Ab	(+)
D-Bil	1.0 mg/dL	HBc-Ab	(+)
AST	37 IU/L	HCV-Ab	(−)
ALT	29 IU/L	AFP	4,014 ng/mL
γ-GTP	391 IU/L	L_3 分画	35.8%
ALP	366 IU/L	PIVKA-II	56 mAU/mL

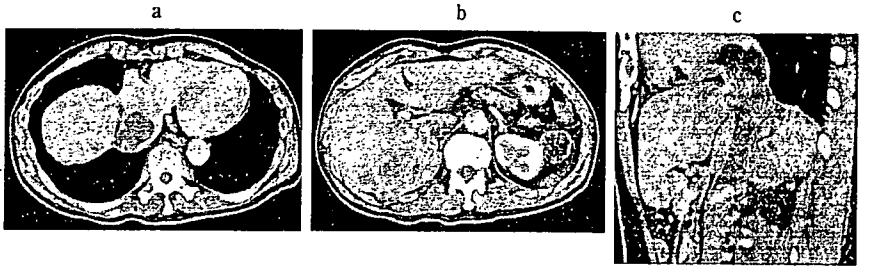

図 1 腹部 CT 検査 (a, b, c)
肝後区域の主腫瘍と肝部下大静脈から右心房内へ進展する腫瘍栓を認める。

図 2 術中所見
肝切除施行後，肝部下大静脈を切開し，右心房内・下大静脈内腫瘍栓を摘出した。

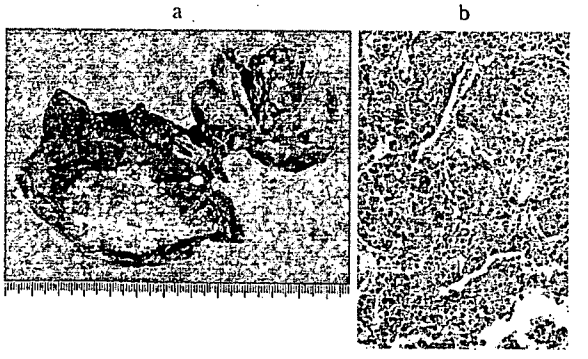

図 3
a: 摘出標本
最大腫瘍径 8 cm．白色の充実性腫瘍で下大静脈内に腫瘍進展を認めた。
b: 病理組織学的所見
HE 染色。低分化型，Edmondson III 型の肝細胞癌の所見であった。

mAU/mL の上昇を認めた（表 1）。

腹部 CT 検査: 肝後区域中心に径 7 cm の主腫瘍と肝部下大静脈をほぼ充満し，さらには右心房内へ進展する腫瘍栓を認めた（図 1）。また右副腎腫大も認め，転移が疑われた。

以上より右心房内腫瘍栓を伴う進行肝細胞癌と診断。2005 年 7 月肝動脈リザーバー留置術の後，doxorubicin/IFN-β 併用化学療法（プロトコールは doxorubicin 10 mg/body＋IFN-β 300 万単位/回の肝動脈内注入を週 3 回，計 4 週間[1]）を 1 クール施行した。抗腫瘍効果は NC であったが，胸部圧迫感などの症状は改善し T-Bil が正常範囲内に復したことより，10 月 17 日に肝拡大後区域切除，右心房内・下大静脈内腫瘍栓摘出，右副腎・横隔膜合併切除，胆嚢摘出術を施行した（図 2）。

摘出標本: 切除肝重量は 442.2 g。原発巣の割面は白色の充実性腫瘍で，最大腫瘍径は 8 cm。原発性肝癌取扱い規約[2]に基づく術後診断は，Ig，Fc（−），Sf（+），massive，S2，N0，Vp2，Vv3，Va0，B0，IM0，P1，SM（−），CH で T3N0M0，Stage III であった（図 3a）。

病理組織学的所見: 術後の病理学的検索では低分化型, Edmondson III型の肝細胞癌で, vp2, vv3, va0, s2, b1, p1, sm(−)(図3b)。背景肝に硬変像はなく, HAI score は Grade 1, Stage 3 であった。

術後経過は特に問題なく退院し, 社会復帰した。外来通院中の2006年1月より肺や骨, リンパ節への遠隔転移を来したため, S-1/IFN-α療法[3,4]を施行したが, 治療効果を認めず, 初回治療から13か月後に癌死した。

II. 考 察

脈管侵襲を伴う肝細胞癌は極めて予後不良である。教室ではこのような進行肝細胞癌に対して, 5-FUの肝動注にIFN-αの皮下投与を併用した化学療法(FU arterial infusion and IFN therapy: FAIT)を機軸とした集学的治療を行い, その良好な成績について報告してきた[5-8]。しかしながら, 過度の腫瘍進展により黄疸や腹水などの肝不全徴候を来したため, 治療適応外となり, 残念ながら緩和医療へと移行せざるを得ない症例も少なからず存在する。このような症例に対しても治療を断念することなく, 予後の改善を図るためには肝不全徴候下にあっても施行し得る何らかの抗腫瘍治療が必要である。

教室では, これまでに *in vitro* でIFN-βと各種抗癌剤の併用による抗腫瘍効果の有用性を報告し[9,10], さらにはパイロットスタディとして, doxorubicin/IFN-β併用化学療法をT-Bilが上昇しているような進行肝細胞癌を対象としてこれまで11例に施行した[11]。本療法においては既報のごとく, たとえT-Bilの上昇があっても肝不全徴候を増強することなく治療の完遂が可能であり, さらにほとんどの症例においてT-Bilの低下など肝機能の改善が得られ, 中間生存期間が12か月と予後の向上を認めた。そこで本症例においてもまず, doxorubicin/IFN-β併用化学療法を施行, 腫瘍進展を抑制し, さらには肝不全徴候の改善後に根治切除を施行することで長期生存を得た。

以上, 既報のパイロットスタディと本症例での経験より, doxorubicin/IFN-β併用化学療法は進行肝細胞癌に対するneoadjuvantとしての可能性を含めた, 集学的治療の有用な選択肢の一つとなり得ると考える。

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