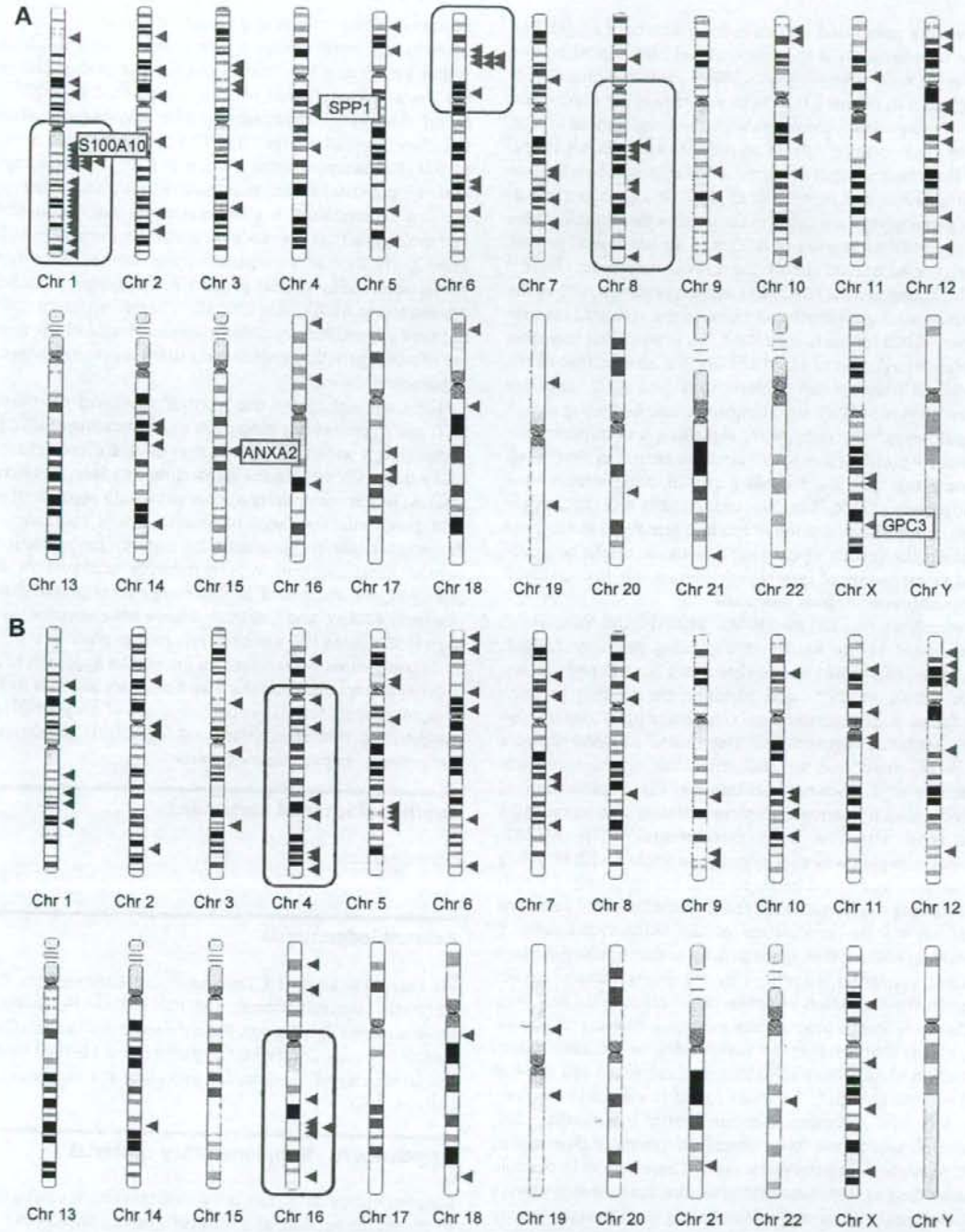

Fig. 5 – Location of 100 upregulated genes (A) and 100 downregulated genes (B) on human chromosomes. Upregulated genes are represented as red arrows, and downregulated genes are represented as blue arrows. Red-coloured circles (chromosomes 1q, 6p and 8q) represent regions with relative concentration of upregulated genes, whilst blue coloured circles (chromosomes 4q and 16q) represent regions with relative concentration of down-regulated genes. ANXA2, S100A10, SPP1 and GPC3 are located at chromosomes 15q21-22, 1q22, 4q21-25 and Xq26.1, respectively.

liver via the portal vein system even in advanced stages, and portal vein invasion is the most crucial histological feature associated with poor prognosis.[3] SPP1 protein expression is upregulated in primary HCC with accompanying metastasis, and SPP1 expression correlates with the invasiveness of HCC cells in tissue culture.[19] Based on network analysis, we speculated that the binding of integrins to SPP1[20] might be related to the progression and metastasis of HCC. GPC3, a heparan sulphate proteoglycan anchored to the plasma membrane, is also a good candidate marker of HCC. It is an oncofoetal protein overexpressed in HCC at both the mRNA and protein levels.[11] We also confirmed that GPC3 was overexpressed in HCC by the immunostaining of paraffin sections. In the activated integrin pathway, GPC3 interacts with IGF-2,[21] a protein that increases the phosphorylation of MAPK1.[22] GPC3 is also related to the zinc-finger transcription factors, GLI1 and GLI2, that are known players in WNT signalling and Sonic hedgehog signalling pathways.[23] Recently, WNT signalling was implicated in hepatocyte proliferation, which could be crucial in liver development, regeneration following partial hepatectomy, and pathogenesis of HCC.[24] In this context, SPP1 and GPC3 might participate in the activation of integrin signalling in HCC and based on the results of clustering analysis, might be implicated as mediators of intrahepatic metastasis, histopathological malignancy or poor prognosis.

Second, we focused on ANXA2, S100A10 and VIM, which were related to the Akt/NF-κB signalling pathway. ANXA2, also called calpactin I heavy chain, is a member of the annexin family of Ca^{2+}- and phospholipid-binding proteins and forms a heterotetrameric complex with S100A10, also called calpactin I light chain.[25] The ANXA2-S100A10 complex has been implicated in the structural organisation and dynamics of endosomal membranes, the organisation of cholesterol-rich membrane microdomains, and connecting lipid rafts with the actin cytoskeleton.[25] The ANXA2-S100A10 complex was also recently associated with recycling endosomes, and might be involved in the recycling of E-cadherin during the formation of the E-cadherin-based adherens junctions via the modulation of the actin cytoskeleton.[26] Moreover, ANXA2 was identified as a Rac binding partner and Rac activation is induced by the interactions of E-cadherin in the formation of adherens junctions.[27] In this way, cadherin–cadherin interactions initiate a cascade of signalling events that result in increased cadherin/Akt association, activation of Akt/NF-κB signalling, and increased cell survival and tumour growth.[27] Akt1 was found to associate structurally with VIM (a structural component of intermediate filaments),[28] which has been found in poorly differentiated HCC as well as hepatoblastomas.[29] Therefore, it is possible that binding of Akt1 and VIM activates downstream players (NF-κB signalling) as well as increasing the intrinsic activity of Akt1. This molecular understanding of HCC progression in Akt/NF-κB signalling was not so different from our result of correlations between clinicopathological features and gene expression profiles. It seems that the higher-activated group in Akt/NF-κB signalling has lower histopathological differentiation.

Currently, the array-based CGH approach is used to study chromosomal aberrations in human cancers. A previously reported meta-analysis[30] showed that the most common chromosomal arms containing gains were 1q, 6p and 8q, whereas the most common losses were found in chromosomes 4q, 8p and 16q. Comparing our expression data with the meta-analysis result of array-based CGH in HCC,[30] we found that our gene expression data surprisingly matched the chromosomal aberrations. The comprehensive analysis of 100 HCC samples using human 30 K DNA microarray revealed a potential association between the global copy number and expression. It is also noteworthy that our identified key molecules that operate synergistically in hepatocarcinogenesis are located at separate chromosomes, so chromosomal aberrations cannot prove a relationship of candidate genes such as ANXA2 and S100A10. Therefore, our integrative network approach can provide a significant clue to the discovery of novel genetic combinations that may be important for hepatocarcinogenesis.

Here, we highlighted the 'hotspot' canonical pathways in HCC and improved our molecular understanding of HCC progression. It is widely recognised that there are distinct molecular subtypes of HCC in the transcriptome space, and current interest of the community spread to include identification of subtype-specific aberration of genes/pathway. This functional genomics study could contribute towards the detection of several signalling pathways commonly activated in HCC. Moreover, we succeeded in detecting two potential disease markers, ANXA2 and S100A10, whose colocalisation in human HCC tissues has not been reported previously.

In conclusion, we reported an integrative approach of genome-wide microarray analysis and network analysis in HCC. This novel approach allows the extraction of deeper biological insight from microarray data and identifying potential key molecules in hepatocarcinogenesis.

Conflict of interest statement

None declared.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.ejca.2008.02.019.

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Original Communications

Comparison of the outcomes between an anatomical subsegmentectomy and a non-anatomical minor hepatectomy for single hepatocellular carcinomas based on a Japanese nationwide survey

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Background. *Although a surgical resection is an important modality for the treatment of hepatocellular carcinoma (HCC), the impact of the operative method on both the patient survival and disease-free survival (DFS) still remains controversial.*

Methods. *Using a nationwide Japanese database, 72,744 patients with HCC who underwent a curative liver resection between 1994 and 2001 were divided into two groups based on whether an anatomical subsegmentectomy (AS) or a non-anatomical minor hepatectomy (MH) was performed. A total of 5,781 patients with single HCCs were selected for the study and divided into 3 subgroups based on the size of the HCCs (less than 2cm, 2 to 5 cm, and greater than 5 cm in diameter). An AS was performed for 2,267 patients while an MH was performed for 3,514 patients.*

Results. *The overall DFS was significantly better after an AS (P = .0089). When the patients were stratified according to the size of the HCC, a better DFS was seen in the patients with HCC from 2 to 5 cm after an AS (P < .0005). Further stratification according to liver damage did not show any significant differences between an AS and an MH.*

Conclusion. *An AS is therefore recommended, especially when the size of HCC ranges from 2 to 5 cm. (Surgery 2008;143:469-75.)*

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This study was performed as a group project by the Liver Cancer Study Group of Japan.

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HEPATOCELLULAR CARCINOMA (HCC) is a common malignancy in Japan, especially in those with virally infected cirrhotic livers.[1] Although an anatomical liver resection is one of the curative treatment options available, a massive anatomical liver resection can be difficult to perform in diseased livers. Only 23% of the patients in the survey of the Liver Cancer Study Group of Japan (LCSGJ) underwent a hemi-hepatectomy.[2] For such cases of HCC in a diseased liver, especially for a single HCC, an anatomical subsegmentectomy (AS) to resect one Couinaud segment was advocated and reported to yield a good outcome.[3]

Another option to treat HCC in a diseased liver is a non-anatomical minor hepatectomy (MH) with a sufficient margin.[4] However, an absolute result of a comparison between an AS and an MH has not been reported, since the previous studies included a resection ranging from an extended hemi-hepatectomy to a subsegmentectomy in an anatomical resection group.[3,4-7] In addition, a comparison between an AS and an MH according to the size of the HCC and the liver function has not yet been fully demonstrated.

LCSGJ has been conducting a nationwide survey of the patients with primary liver carcinoma since 1969 in order to evaluate the epidemiologic and clinical characteristics, histopathologic features, diagnosis, treatment modalities, and outcomes.[2] Using the large-scale registration system of the LCSGJ, we herein evaluated the outcomes of an AS and an MH in order to clarify the impact of these two treatments on the survival of patients with a single HCC, and to identify which patients can benefit from these resections.

MATERIALS AND METHODS

Patients. In a nationwide follow-up survey of primary HCCs conducted by LCSGJ, patients with primary malignant liver tumors who had been diagnosed by imaging studies, preoperative clinical data, and/or histopathologic studies at approximately 795 institutions in Japan were registered and followed prospectively every two years. This database contained 92,369 patients who were diagnosed with liver tumor and 72,744 patients who were finally diagnosed with HCC between 1994 and 2001. From this group, 5,781 patients with a single HCC who had undergone either an AS (n = 2,267) or an MH (n = 3,514) were enrolled. The indications for a hepatic resection and the types of operative procedures were usually determined based on the patients' liver functional reserved, mainly assessed by Makuuchi Criteria, which comprise preoperative measurements of ascites, serum bilirubin level, and indocyanine green retention rate at 15 minutes.[8] This nation-wide study was prospectively performed but not randomly allocated, so this study is an "As treated" analysis and not an "Intention to treat" analysis.

Operative methods. In an AS, accurate determination of the resection area, i.e. one Couinaud segment, was typically performed with an intraoperative ultrasound and the complete resection of the portal territory containing the tumor was accomplished using either a dye puncture or the Glissonean method.[9,10] In a non-anatomical MH, a surgical margin from the tumor edge of at least 5 mm was secured whenever possible.[11] When it was not possible, however, liver parenchymal transection was performed without exposing the tumor surface, allowing the enucleation of the tumor. In all patients, the resection surface was histologically found to be free from HCC. Usually the liver parenchyma was divided with an ultrasonic dissector or the clamp-crushing method. The operation allocation depended on the policy of each surgeon in each facility. The median follow-up period was 789 days (range, 2 to 5860 days), the mean patient age was 63.1 years, and the male/female ratio was 76:24 (Table I). The patients were stratified according to the size of a single HCC. Specifically, the patients were divided into three subgroups; HCCs less than 2cm, HCCs 2 to 5 cm, and HCCs greater than 5 cm in diameter. In addition, the patients were further stratified according to the degree of liver damage, a procedure which was advocated by the LCSGJ as an alternative to the Child–Pugh score, which was defined by preoperative measurements of ascites, serum bilirubin level, serum albumin level, indocyanine green retention rate at 15 minutes, and prothrombin activity (Table II).[12] All recurrences were documented using computed tomography and/or magnetic resonance imaging with contrast media.

Statistics. The patient survival and the disease-free survival (DFS) curves of the AS and PH cases were generated using the Kaplan-Meier method and then were compared by the log-rank test. To investigate the hazard ratio of AS to MH, the Cox regression model was used. The major event of the model regarding patient survival was the cancer death of the patients, while for the DFS model it was the recurrence of HCC. *P* values of less than .05 were considered to be statistically significant. To accurately compare the effects of an AS with an MH, the outcomes were measured using the DFS because the patient survival after a recurrence could be affected by the subsequent treatment at other facilities.

Table I. Patient demographics

	Operative methods					
	AS		MH		Total	
	N = 2267		N = 3514		N = 5781	
	n	(%)	n	(%)	n	(%)
Gender						
Male	1764	77.81	2619	74.53	4383	75.82
Female	502	22.14	894	25.44	1396	24.15
unknown	1	0.04	1	0.03	2	0.03
Age (years old)						
N	1955		3079		5034	
Mean	62.7		63.4		63.1	
SD	9.23		9.02		9.11	
SE	0.21		0.16		0.13	
unknown	312		435		747	
Tumor size						
N	2213		3429		5642	
<=2cm	622	28.1	1457	42.5	2079	36.8
2< <5cm	1412	63.8	1798	52.4	3210	56.9
>=5cm	179	8.1	174	5.1	353	6.3
Liver Damage						
N	2163		3286		5449	
unknown	104		228		332	
A	1615	74.7	2121	64.5	3736	68.6
B	530	24.5	1100	33.5	1630	29.9
C	18	0.8	65	2.0	83	1.5
Observation period (days)						
N	2267		3514		5781	
Mean	951.6		948.2		949.5	
SD	761.38		744.50		751.10	
SE	15.99		12.56		9.88	
Operative mortality	16	0.71	30	0.86	46	0.79

Table II. Degree of Liver Damage Classified by the Liver Cancer Study Group of Japan

Item	Degree of liver damage		
	A	B	C
Ascites	none	Controllable	Uncontrollable
Serum bilirubin (mg/dl)	<2.0	2.0-3.0	>3.0
Serum albumin (g/dl)	>3.5	3.0-3.5	<3.0
ICGR15(%)	<15	15-40	>40
Prothrombin activity (%)	>80	50-80	<50

NOTE. If 2 or more items scoring the same grade occurred in the 2 grades, the higher grade is adopted as the degree of liver damage. ICG R15(%), indocyanine green retention rate at 15 minutes

RESULTS

Table I shows the operative mortality by an AS and an MH as 0.71% and 0.86%, respectively. Fig 1 shows the overall survival of all patients with single HCCs treated either with an AS or an MH. The patient survival seemed to be better after an AS than after an MH but without statistical significance (hazard ratio 1.120, $P = 0.0531$). The DFS was significantly better after an AS than after an MH (hazard ratio 1.121, $P = .0089$).

After stratification according to the size of a single HCC, it was found that the HCCs measuring less than 2 cm in size numbered 2,079, HCCs ranging from 2 to 5 cm in size numbered 3,210, and HCCs greater than 5 cm in size totaled 353 cases, (size unknown 139 cases). When the survival was stratified according to above-mentioned size of HCC, the DFS was better after an AS as compared with the DFS after an MH for HCCs in the 2 to 5 cm diameter range (hazard ratio 1.216, $P = .0005$,

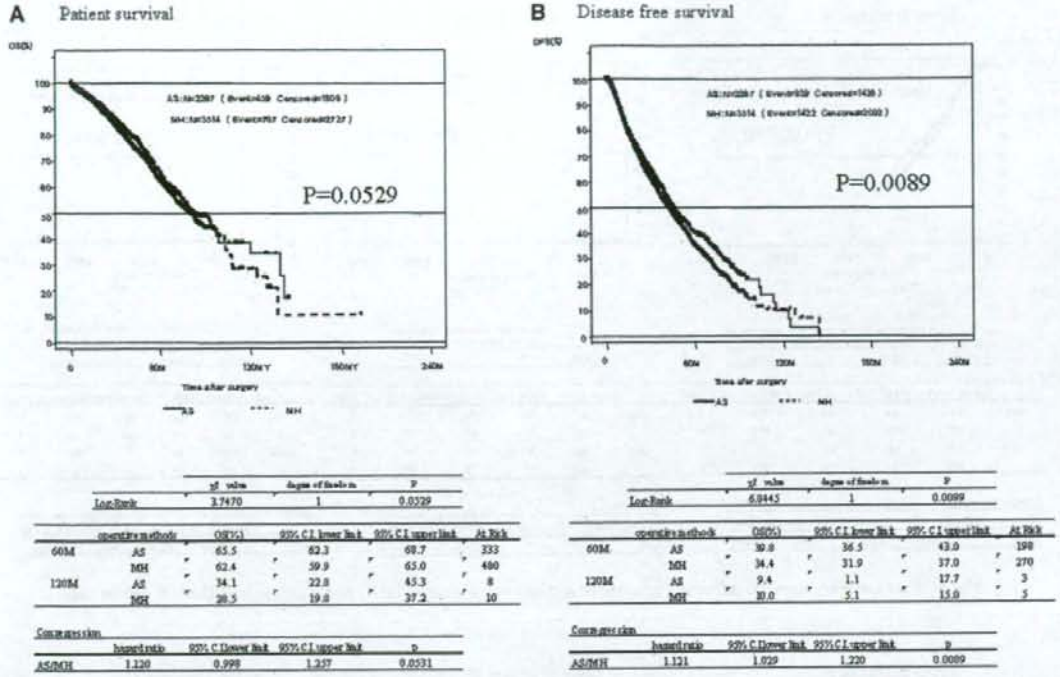

Fig 1. Survival rate after a curative resection for a single HCC. **A,** Patient survival; **B,** Disease free survival (DFS). C.I., confidence interval.

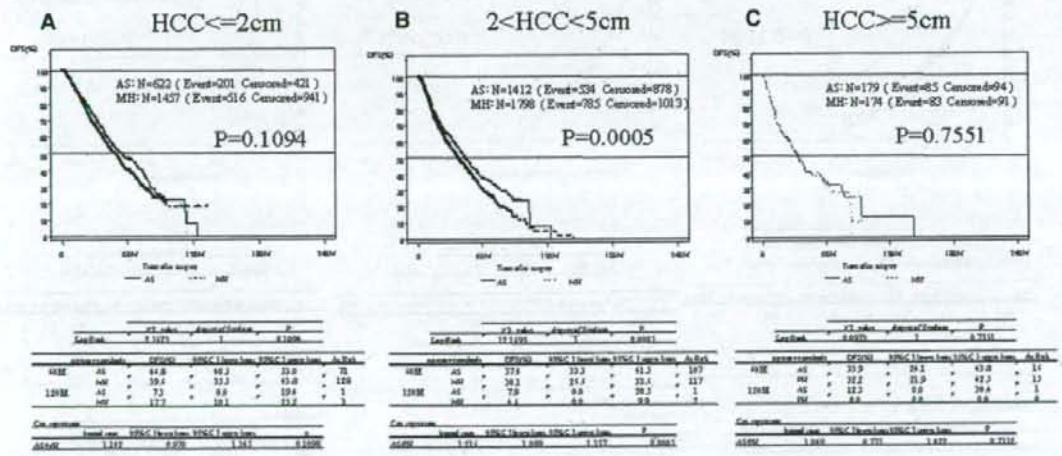

Fig 2. Disease free survival after a curative resection for a single HCC. **A,** HCC less than 2cm; **B,** HCC between 2 to 5cm; **C,** HCC larger than 5 cm.

Fig 2). However, for HCCs less than 2 cm and greater than 5 cm in diameter, the DFS was not significantly different between the two treatment groups (Fig 2).

When HCC patients were further stratified according to the degree of liver damage, the differences in the DFS rates between the AS and MH groups, such as those seen in the 2-5 cm diameter HCC group, disappeared (Figs 3, 4, 5). In Table I, not many HCCs larger than 5cm in size were indicated for either AS or MH (n = 353, 6.3%), since they are usually treated with

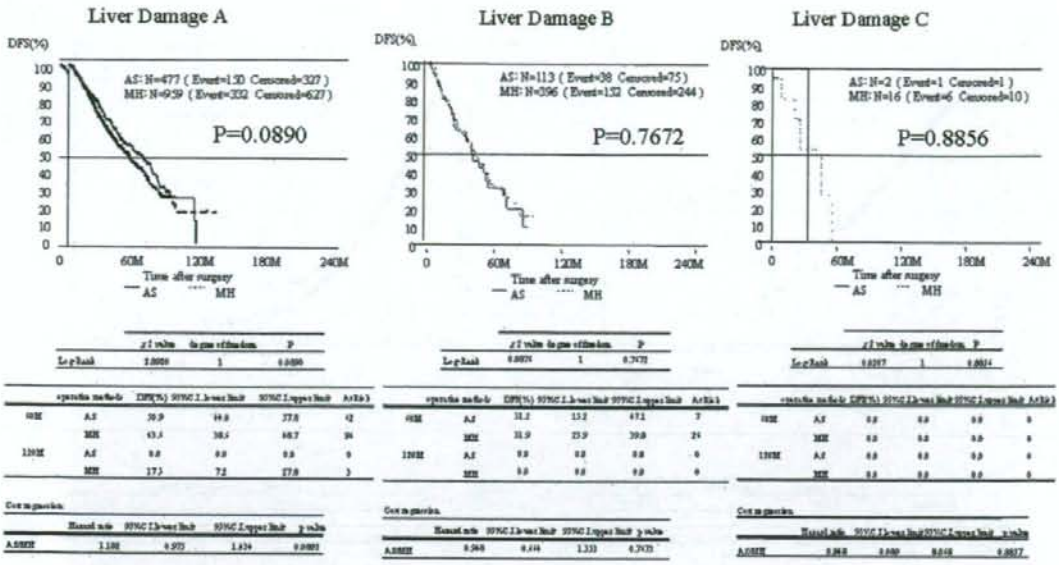

Fig 3. Disease-free survival after a curative resection for a single HCC measuring less than 2 cm in size.

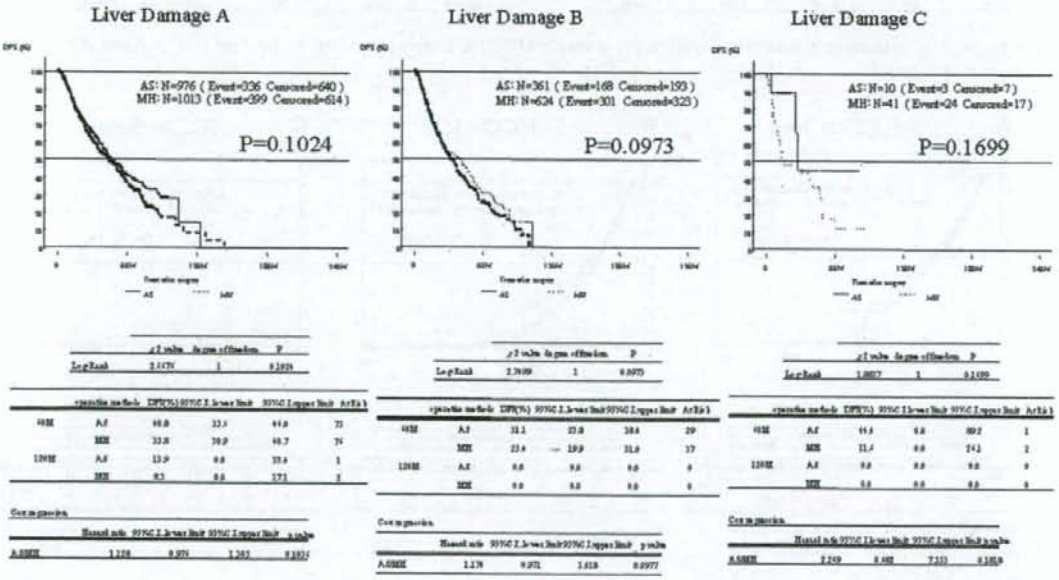

Fig 4. Disease-free survival after a curative resection for a single HCC of 2 to 5 cm.

extensive resections. In addition, the HCCs which developed in a Liver Damage C liver (n = 83, 1.5%), even those measuring less than 2cm in diameter, were not indicated for either AS or MH due to fear of inducing postoperative liver failure.

DISCUSSION

Using this large nationwide registration for HCC in Japan, we herein have demonstrated that an AS yielded a better DFS than did an MH for all single HCCs, especially for a tumor

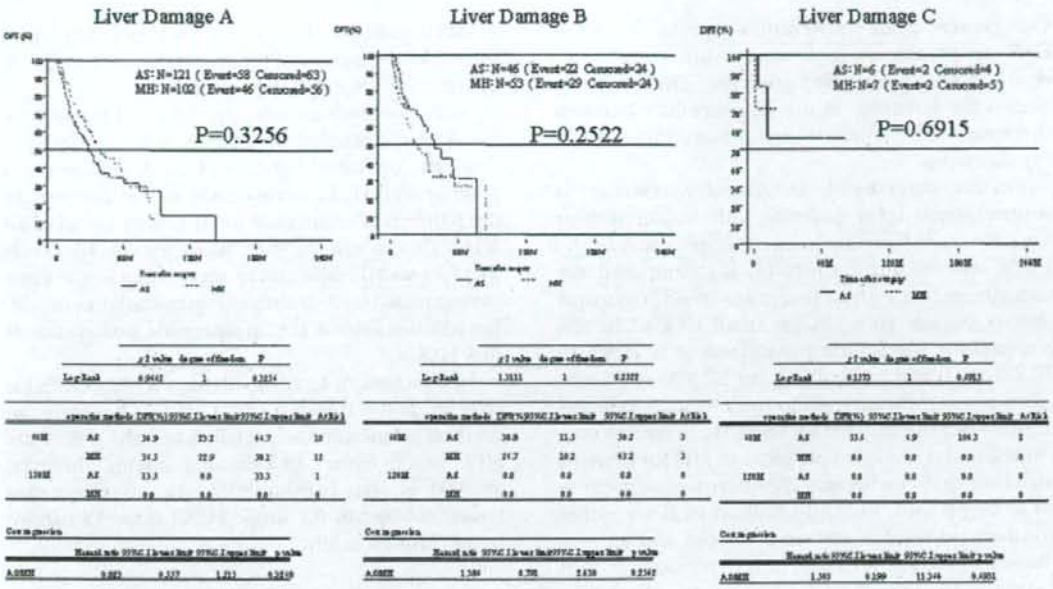

Fig 5. Disease-free survival after a curative resection for a single HCC larger than 5 cm in size.

between 2 and 5 cm in diameter. Although there was no statistical difference in the patient survival after an AS or an MH, it is important to note that, in this large scale study, even the patient survival only demonstrated a very slight statistically significant difference.

It seems reasonable that there was no statistical difference in the DFS between an AS and an MH because HCCs of less than 2 cm are generally effectively treated with other modalities such as local ablation therapy. In addition, for HCCs larger than 5 cm, an MH was comparable to an AS, because the stage of the HCC is elevated, and the oncological behavior of HCC is evident, and may offset the effects of the operative methods. Therefore, an AS became a strong prognostic factor, especially for HCCs measuring from 2 to 5 cm in size. In fact, the statistical probability of an AS on the DFS is greater for HCCs of measuring 2 to 5 cm in size ($P = .0005$) in comparison with that seen in all HCCs before stratification ($P = .0089$). It is also noteworthy to mention that the percentage of liver damage in patients within this group was not statistically higher than that occurring in the other groups (2 cm> HCC, 73.2%; 2 cm <HCC <5 cm, 65.6%; 5 cm<HCC, 66.6%). Namely, the liver function could not account for the improved outcomes in the 2 cm <HCC <5 cm group. For the patients with a preserved liver function, as in Child-Pugh A, a liver resection is a viable option for HCC treatment. For this group of patients, various reports on the benefit of an AS or an MH show mixed results. In 1999 Nagasue et al[13] analyzed the outcome of extended (more than 2 subsegments) and partial liver resections for patients with HCCs less than 10 cm with preserved liver function (Child–Pugh A). They found no significant difference in the patient survival and the DFS between the methods of resection. On the other hand, in 2002, Regimbeau et al compared the outcomes of a curative MH in HCCs with at least 1 cm of surgical margin and of the anatomical liver resections for Child-Pugh A cirrhotic patients with HCCs measuring less than 4 cm in diameter.[5] The five- and eight-year patient survival rates were better in the anatomical resection group than in the MH group (five-year patient survival of 35% vs. 54%, eight-year patient survival of 6% vs. 45%), and the five- and eight-year DFS rates were also better in the anatomical resection group (26% vs. 45%, 0% vs. 21%). There were more local recurrences in the PH group. In 2004, Hasegawa et al[3] compared the outcomes of the anatomical and non-anatomical resections for a single HCC in Child-Pugh A and B groups (83% of the patients were Child-Pugh A) in a single center. The five-year patient survival and the DFS rates were better in the anatomical resection group (patient survival, 66% vs. 35%; DFS, 34% vs. 16%). In a multivariable analysis, an anatomical resection was one of the prognostic factors.

Our present study showed outcomes of the AS in DFS comparable to their study, while patient survival was not statistically significant. This might be due to the variability of the AS procedure between the nearly 800 Japanese institutions included in the database.

On the other hand, an extended resection is contraindicated for patients with impaired liver function, found mostly in cirrhotic patients. In the 1980s, one of our authors (T. K.) compared the outcome of limited liver resections (n = 37) to major liver resections (n = 13) for small HCCs.[4] In this comparison, the patient survival was only 79.9% to 78.7% at 1 year, and 32.6% to 22.5% at 5 years (thus not significant), while the mortality rate was higher after the major resections. We therefore concluded that it is valid to perform an MH for patients with liver cirrhosis because they have a poor prognosis to begin with. Recently, Kaibori et al[6] reported comparable results between a limited and an anatomical resection for HCC in a cirrhotic liver with hepatitis C. Therefore, although an AS should be performed to increase the survival of patients with HCC, an MH can be considered if an AS is not appropriate, especially for patients with poor liver function.

In this study, all resection margins were confirmed to be histologically free from HCC. In other words, only R0 (histologically curative) resections were included. However, no data regarding the distance from the resection margin and HCC were obtained in the LCSGJ study. Basically, this study was performed to see whether a resection of same portal territory of HCC (i.e. subsegment, namely, Couinaud one segment) has an impact on the disease free survival in a whole nation study in Japan rather than to clarify the impact of the distance from HCC to cut surface.

In the present study, there were no significant differences when the patients who underwent an AS or an MH were further stratified according to the degree of liver damage that is close to the Child-Pugh score, even in patients with HCCs of 2 to 5 cm in diameter. This might have been due to the decrease in the number of patients in each group of liver damage. For example, in patients with HCCs of 2 to 5 cm in diameter, 5 and 10 year survival rate after AS and MH were 37.4, 7.8% and 30.1, 4.6% with *P*-value of 0.0005 in Fig 2. When these patients were stratified with liver damage, 5 and 10 year survival rate after AS and MR were 40.8, 13.9% and 33.8, 8.3% with *P*-value of .1024 in patients with liver damage A (n = 1989). It is quite possible that this statistical insignificance will be changed with increase in sample size. However, there is still a possibility that original cohort of 3210 patients (HCCs 2-5cm) might have produced an overpowered study with statistical significance but little clinical relevance.

Since our present study provides Level 2a evidence using a large number of patients, it is considered to show the operative benefits of an AS for selected patients with HCC. In this study we did not analyze the patterns of recurrence of HCC after an AS or an MH. This needs further investigation to clarify whether an MH yields more recurrence in the same subsegment (i.e. intrahepatic metastasis) or in different subsegments (i.e. multicentric occurrence of new HCC).

In conclusion, an AS resulted in a better DFS for selected patients with a single HCC. Therefore, an AS is recommended, especially when the size of the HCC ranges from 2 to 5 cm in diameter. However, an MH is also considered to be an alternative treatment option for single HCC, if an AS cannot be performed safely.

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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 ≥1000/mm³, platelet count ≥40 000/mm³, 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.

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.

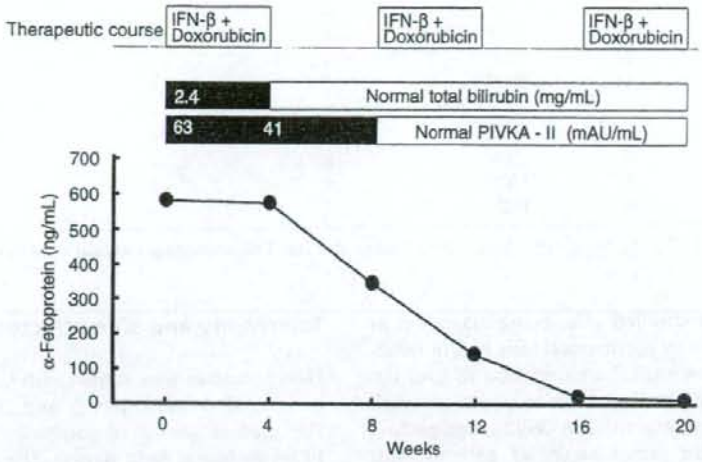

Figure 1 Time course of tumor markers in a complete remission case. A 63-year-old man with diffused type advanced hepatocellular carcinoma (HCC) (no. 8) was treated with three courses of combined β-interferon (IFN-β) and doxorubicin intra-arterial injection therapy without severe side-effects. Serum 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)

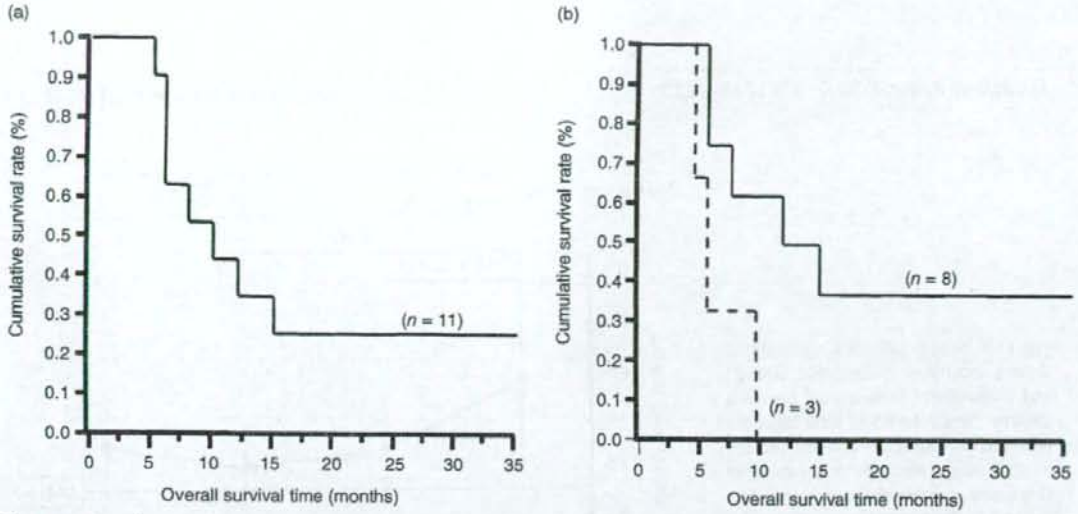

Figure 2 Overall survival periods of patients with advanced hepatocellular carcinoma who received combined β-interferon (IFN-β) and doxorubicin intra-arterial injection therapy. (a) Overall survival periods of 11 patients who received combined IFN-β and doxorubicin intra-arterial injection therapy. The 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.

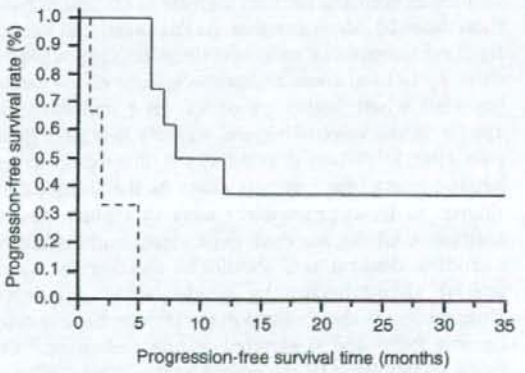

Figure 3 Progression-free survival times of patients with advanced hepatocellular carcinoma according to responses to β-interferon and doxorubicin combination therapy. One-year survival rate for CR or SD patients was 62.5% (5/8) and that for PD was 0% (0/3). The progression-free survival time for CR 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

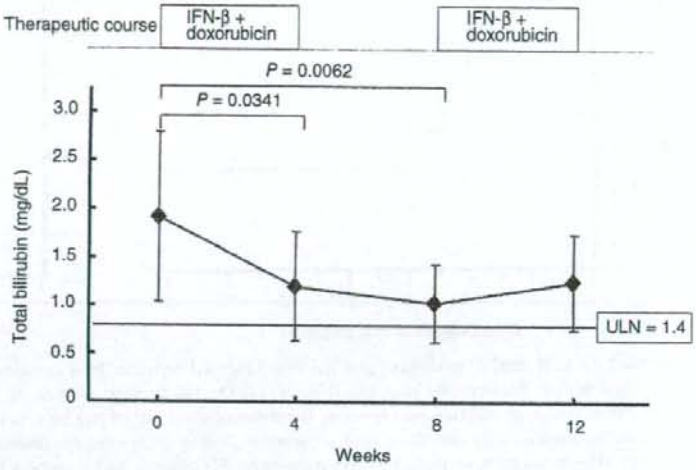

Figure 4 Serum bilirubin ameliorated during combined β-interferon (IFN-β) and doxorubicin intra-arterial injection therapy. Serum levels of total bilirubin decreased significantly and entered the normal range after the first course of combined chemotherapy, and remained in the normal range during the 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

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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Surgical resection vs. percutaneous ablation for hepatocellular carcinoma: A preliminary report of the Japanese nationwide survey☆

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See Editorial, pages 502–504

***Background/Aims*: The treatment of choice for HCC remains controversial. We evaluated the therapeutic impact of surgical resection, PEI, and RFA for HCC on outcomes.**

***Methods*: A database derived from a Japanese nationwide survey of 17,149 patients with HCC treated by resection, PEI, or RFA between 2000 and 2003 was used to identify 7185 patients with no more than 3 tumors (≤3 cm) and a liver function of Child-Pugh class A or B. The patients classified into either a resection ($n = 2857$), RFA ($n = 3022$), or PEI group ($n = 1306$) and their long-term outcomes were compared.**

***Results*: The median follow-up period was 10.4 months. The 2-year time-to-recurrence rate was 35.5%, 55.4%, and 73.3% in the resection, RFA, and PEI groups, respectively, while the number of recurrences was 2410, 2368, and 862. Although the number of deaths was 55 (1.9%), 49 (1.6%), and 39 (3.0%), the overall survival rates were not different. In a multivariate analysis, surgical resection was a significant negative factor for recurrence as compared with RFA (relative risk, 0.62 [95% confidence interval, 0.54–0.71], $P < 0.0001$) and PEI (0.45 [0.38–0.52], $P < 0.0001$).**

***Conclusions*: This preliminary report suggested that surgical resection may provide less time-to-recurrence rate than either RFA or PEI in patients with HCC.**

***Keywords*: Hepatocellular carcinoma; Surgical resection; Radiofrequency ablation; Percutaneous ethanol injection; Nationwide survey**

1. Introduction

Recent advances in the diagnosis and treatment of hepatocellular carcinoma (HCC) have improved short- and long-term outcomes [1]. Many treatments are now available for HCC, such as surgical resection, percutaneous ablation, and transcatheter hepatic arterial chemoembolization (TACE). Treatment for individual patients is selected on the basis of the stage of liver disease and tumor status, but clear-cut guidelines are lacking.

Since 1965, the Liver Cancer Study Group of Japan has conducted nationwide surveys of patients with HCC. In

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Abbreviations: HCC, hepatocellular carcinoma; RFA, radiofrequency ablation; PEI, percutaneous ethanol injection; TACE, transcatheter hepatic arterial chemoembolization.

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2000, we reported the results of a study comparing survival after surgical resection, percutaneous ethanol ablation (PEI), and TACE, based on a large database including 30,000 patients registered between 1988 and 1996 [2]. Surgical resection was found to result in longer survival than PEI in clinical stage I [3] disease with a single tumor (≤5 cm) and in clinical stage II disease with a single tumor (>2 cm but ≤5 cm) or 2 tumors (≤2 cm). TACE was inferior to both surgical resection and PEI. On the basis of these results, "Clinical practice guidelines for hepatocellular carcinoma", published in 2005 [4], recommended surgical resection and percutaneous ablation as first-line treatment and second-line treatment, respectively, in patients with good liver function, classified as liver damage A or B [3], and a single tumor or no more than 3 tumors each measuring ≤3 cm in diameter.

In the early 1990's, radiofrequency ablation (RFA) was introduced [5] and has since been widely used in place of PEI [6–8]. The results of randomized controlled trials have recently confirmed that RFA has better outcomes than PEI [9,10]. In view of these findings, the superiority of surgical resection over percutaneous ablation, supported by the results of our previous study comparing surgery with PEI [2], should now be re-evaluated by comparing surgery with RFA. We therefore compared long-term outcomes after surgical resection, RFA, and PEI based on data obtained in the latest Japanese survey.

2. Patients and methods

With the cooperation of 795 institutions in Japan, patients with primary liver cancer have been registered every 2 years and followed up prospectively in a nationwide survey conducted by the Liver Cancer Study Group of Japan. HCC was diagnosed on the basis of imaging studies, clinical data, and/or histopathological studies at each institution. Between 2000 and 2003, a total of 17,149 patients who underwent surgical resection, RFA, or PEI as a primary treatment for HCC were newly registered. Since 2000, RFA began to be more widely used in Japan, making the study period suitable for the assessment of this treatment. We studied outcomes in 7185 patients who met the following criteria, which are consistent with a common indication for RFA and PEI [10,11]: (1) no more than 3 tumors (≤3 cm in diameter) and (2) a liver function of Child-Pugh class A or B. Patients for whom outcome data were not available were excluded. The 7185 patients were classified according to primary treatment into a resection group ($n = 2857$), RFA group ($n = 3022$), and PEI group ($n = 1306$).

The patients were prospectively followed up at each institution. There is no definite follow-up protocol, however, most of the hepatologists and liver surgeons have observed the protocol, as shown in "Clinical practice guidelines for hepatocellular carcinoma" [4], in which ultrasonography and measurement of the tumor markers every 3 or 4 months and enhanced computed tomography or magnetic resonance imaging every 6 or 12 months is recommended.

The relevant clinical data were collected and analyzed. The baseline characteristics of the three groups (Table 1) were compared by analysis of variance for continuous variables, and by chi-square or Mantel-trend tests for categorical variables. The resection group had higher proportions of younger patients and male patients than did the RFA and PEI groups. Hepatitis C virus infection was less prevalent in the resection group than in the RFA and PEI groups. On the basis of Child-Pugh class, serum albumin and total bilirubin levels, platelet counts, and the indocyanine green retention rate at 15 min, liver function was better in the resection group than in the RFA and PEI groups. As for tumor-related factors, the number of tumors was smaller and the maximum tumor diameter was larger in the resection group than in the RFA group or PEI group. The resection group had the lowest proportion of patients with an abnormally elevated alpha-fetoprotein level (≥15 ng/mL) and the highest proportion of patients with an abnormal des-γ-carboxy prothrombin level (≥40 AU/mL).

Overall survival and time-to-recurrence curves were plotted by the Kaplan–Meier method and compared by the log-rank test. Recurrence was diagnosed on the basis of imaging studies, clinical data, and/or histopathological studies at each institution. When calculating time-to-recurrence, death without recurrence was defined as a censor.

The therapeutic impact of surgical resection, RFA, and PEI was estimated using a Cox proportional-hazards model, including the following 9 covariates: age, gender, Child-Pugh class, hepatitis C virus antibody, platelet count, tumor number, tumor size, alpha-fetoprotein level, and des-γ-carboxy prothrombin level. The results of multivariate analysis are expressed as relative risks with 95% confidence intervals. P values of <0.05 were considered to indicate statistical significance.

3. Results

The median follow-up period after treatment was 10.4 months, and the 25th percentile and 75th percentiles were 4.8 and 16.7 months, respectively. Overall survival rates at 1 and 2 years in the resection group, RFA group, and PEI group were 98.3%/94.5%, 98.5%/93.0%, and 98.2%/92.3%, respectively (Fig. 1). The differences between the groups were not significant. The number of deaths in the resection, RFA, and PEI groups was 55 (1.9%), 49 (1.6%), and 39 (3.0%), respectively. Time-to-recurrence rates at 1 and 2 years in the resection, RFA, and PEI groups were 17.0%/35.5%, 26.0%/55.4%, and 32.5%/73.3%, respectively, while the number of recurrences was 2410, 2368, and 862, respectively. The time-to-recurrence rate was significantly lower in the resection group than in the other two groups (Fig. 2, $P < 0.0001$).

The multivariate analysis showed that the impact of treatment on overall survival did not differ significantly among the three groups (Table 2). Low tumor marker levels (alpha-fetoprotein <15 ng/mL and des-γ-carboxy prothrombin <40 AU/mL), tumor size <2 cm, better liver function (Child-Pugh class A), and the presence of hepatitis C virus infection were favorable factors for overall survival. Among the 3 treatments, surgical resection had the greatest negative impact on recurrence, because relative risk as compared with RFA and PEI was 0.62 (95% confidence interval; 0.54–0.71, $P < 0.0001$) and 0.45 (0.38–0.52, $P < 0.0001$), respectively. A single tumor, low tumor marker levels, a small tumor size, the absence of hepatitis C virus infection, and younger age were negative factors for recurrence.

4. Discussion

In this study, time-to-recurrence rate was lower after surgical resection for HCC than after RFA or PEI, and