Figure 5. siRNA for survivin and CDDP induce apoptosis. Western blot analysis of cleaved caspase-3 (A), cleaved PARP (B), phospho-Akt (C) and XIAP (D) in HCC cells, 24 h after treatment. Data are expressed as the mean ± SEM (n=4).

There was little survivin expression in non-tumor liver tissues from rat treated with DEN. Overexpression of survivin occurs frequently during cellular promotion of malignant neoplasms and has been identified as a negative prognostic factor in various cancer types (8,14,16). In our study, suppression of survivin expression in rat HCC cells treated with siRNA for survivin attenuated cell proliferation of cancer cells. Survivin was overexpressed in rat HCC cells treated with CDDP, which was down-regulated by treatment with siRNA for survivin. Furthermore, siRNA for survivin sensitized rat HCC cells to CDDP-induced apoptosis. Our data show that CDDP or siRNA for survivin induced apoptosis in rat HCC cells, and that expression of survivin is regulated by the PI3K/Akt pathway, which has recently emerged as being frequently activated in various cancer types. Our results concur with previous reports. Olie *et al* demonstrated that targeting survivin has the potential to induce apoptosis and to increase the sensitivity to chemotherapy in lung cancer cells (28). Yang *et al* demonstrated that siRNA targeting survivin has the potential to increase the sensitivity of drug-resistant lung

cancer cells to anticancer drugs (29). On the other hand, our results indicated that XIAP, one of the major members of the IAP families, was not involved in the regulation of survivin. It has been reported that survivin may block apoptosis by inhibiting caspase-3 and caspase-7 activities directly (5,30). We also identified increased cleaved caspase-3 levels, which indicates activation of caspase-3, in cells treated with siRNA for survivin.

Akt is a key mediator of the PI3K/Akt signaling pathway and is located at an intersection of multiple pathways that have been implicated in cell proliferation, survival, transcription and metabolic processes (31). Inhibition of the PI3K/Akt signaling pathway using the PI3K inhibitor (LY 294002) blocks growth and induces apoptosis (32). While the activity of Akt is tightly linked to PI3K activation, other factors can directly act on Akt to stabilize its oncogenic function. Previous studies have demonstrated that survivin expression was up-regulated in the human HCC cell line, Hep3B, with CDDP treatment (33,34). Furthermore, we demonstrated that CDDP up-regulated survivin expression in the rat HCC cell line
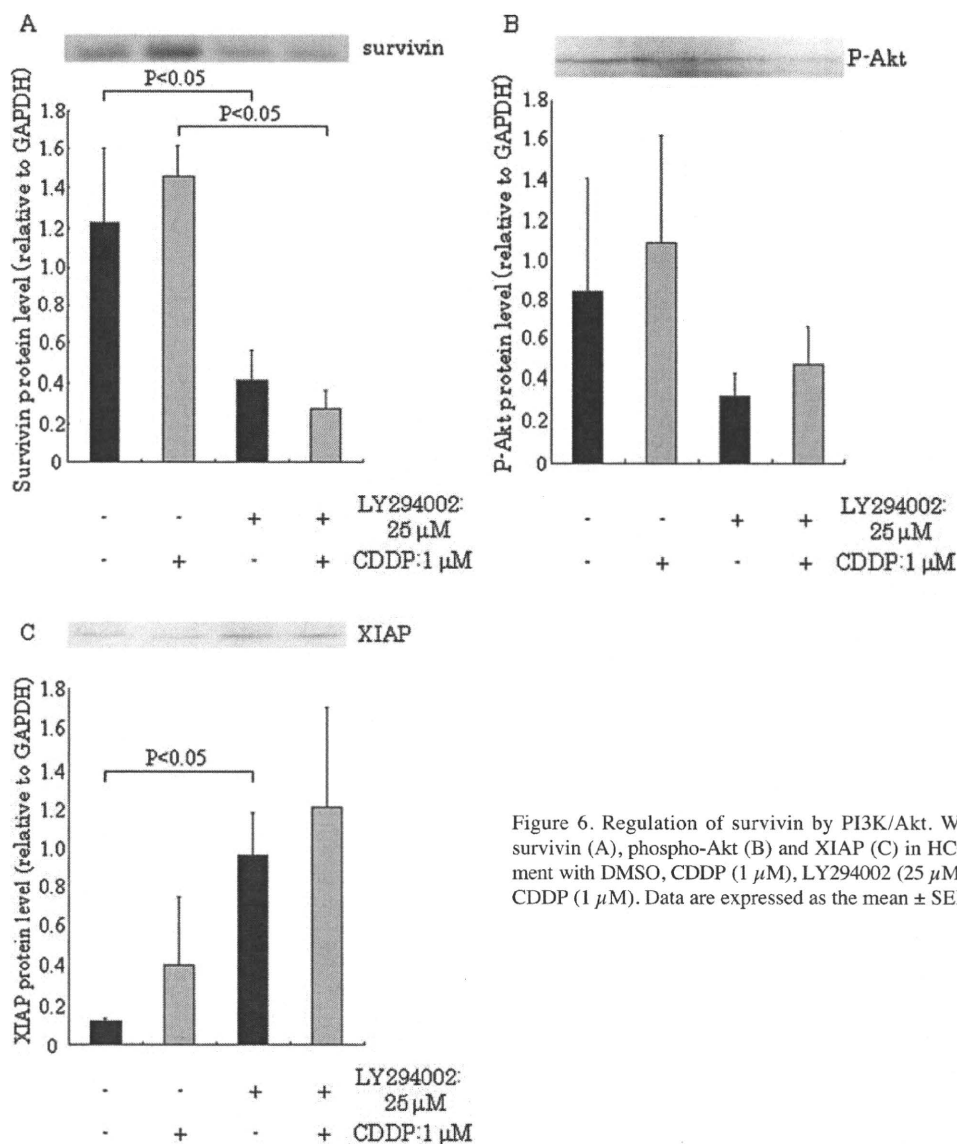
Figure 6. Regulation of survivin by PI3K/Akt. Western blot analysis of survivin (A), phospho-Akt (B) and XIAP (C) in HCC cells, after 24 h treatment with DMSO, CDDP (1 μM), LY294002 (25 μM), LY294002 (25 μM) + CDDP (1 μM). Data are expressed as the mean ± SEM (n=4).

K-251 and that CDDP also activated Akt, resulting in increased survivin and p-Akt levels. Inhibition of the PI3K/Akt pathway decreased overexpression of survivin induced by CDDP and sensitized HCC to CDDP. Therefore, we concluded that survivin is regulated by the PI3K/Akt pathway and that inhibition of survivin expression with siRNA sensitized HCC cells to CDDP-induced apoptosis via the PI3K/Akt-dependent pathway. However, whether PI3K/Akt up-regulates survivin directly, or indirectly remains to be investigated.

We demonstrated that overexpression of survivin and its anti-apoptotic activity in rat HCC cells were regulated by PI3K/Akt signaling. These findings have significant clinical implications, since they provide direct evidence that CDDP itself can trigger resistance in HCC cells which can diminish its therapeutic efficacy. However, inhibition of the PI3K/Akt pathway may have clinical limitations, because of their essential function in all types of cells in a complicated largely unresolved signaling network, which would reduce tumor-specificity. Our results demonstrated that survivin-specific siRNA down-regulates the overexpression of survivin induced by CDDP, and sensitize HCC cells to CDDP-induced apoptosis. The trans-catheter arterial chemo-embolization therapy

has already been applied for practical use, and we previously reported that hydro-administration of an siRNA expressing plasmid into the hepatic artery was a valid method for targeting rat HCC (26). Combination therapy of trans-catheter arterial chemo-embolization and siRNA for survivin, or hydro-administration injection and siRNA for survivin may be beneficial in the treatment of HCC. Furthermore, non-cancerous liver tissues may not responce to siRNA for survivin because they do not endogenously express survivin. Therefore, down-regulation of survivin has the potential to enhance the efficacy of chemotherapy in the treatment of HCC and may have significant clinical impact.

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Original Article

Comparative study of cisplatin and epirubicin in transcatheter arterial chemoembolization for hepatocellular carcinoma

Kenya Yamanaka,[1] Etsuro Hatano,[1] Masato Narita,[1] Kojiro Taura,[1] Kentaro Yasuchika,[1] Takashi Nitta,[1] Shigeki Arizono,[2] Hiroyoshi Isoda,[2] Toshiya Shibata,[2] Iwao Ikai,[1] Tosiya Sato[3] and Shinji Uemoto[1]

Departments of [1]Surgery and [2]Diagnostic Imaging and Nuclear Medicine, Graduate School of Medicine, Kyoto University, and [3]Department of Biostatistics, Kyoto University School of Public Health, Kyoto, Japan

Aim: Transcatheter arterial chemoembolization (TACE) is an established treatment for unresectable hepatocellular carcinoma (HCC). However, it is unclear which chemotherapeutic agent should be selected for TACE. The aim of this study was to compare the efficacy of cisplatin (CDDP) with that of epirubicin (EPI) in TACE for patients with unresectable or relapsed HCC.

Methods: We performed a historical cohort study involving 131 patients treated with a first TACE, defined as either an initial treatment for previously untreated HCC or a first treatment for relapsed HCC after curative resections or ablations. Efficacy was estimated as the response rate (RR) and it was adjusted for the confounding factors that were defined in this study.

Results: The RR were 62.5% (20/32) for the first TACE with CDDP and 51.5% (51/99) for that with EPI. In the adjusted analysis for a history of hepatectomy, percutaneous treatment combined with TACE and tumor factors, the odds ratio was 1.72 (95% confidence interval [CI] = 0.70–4.48). However, a test for interaction between the number of tumors and the chemotherapeutic agent was statistically significant ($P = 0.016$). In multiple HCC, the RR were 66.7% (10/17) for CDDP and 39.6% (30/46) for EPI. The odds ratio was 4.11 (95% CI = 1.14–17.2).

Conclusion: CDDP may be more effective than EPI in TACE for multiple HCC. A randomized controlled study is needed to clarify the efficacy of CDDP in TACE in patients with multiple HCC.

Key words: cisplatin, epirubicin, hepatocellular carcinoma, transcatheter arterial chemoembolization, treatment

INTRODUCTION

EVERY YEAR, AT least 626 000 people die of hepatocellular carcinoma (HCC).[1] Even if a curative resection is performed, 80% of patients develop an intrahepatic recurrence because of either intrahepatic metastases from the primary tumor or multicentric carcinogenesis.[2] Therefore, a therapeutic strategy against advanced or relapsed tumors is vital for improving the prognosis of HCC.

Transcatheter arterial chemoembolization (TACE) provides a survival benefit in patients with unresectable or relapsed HCC.[3–6] Chemotherapeutic agents currently used for TACE are doxorubicin, epirubicin (EPI), mitomycin and cisplatin (CDDP).[7] Furthermore, doxorubicin-loaded drug-eluting beads (DEB) have been recently used for TACE.[8,9] However, ischemia resulting from embolization may be the main factor inducing a reduction in tumor size after TACE.[7] Therefore, it is unclear whether the selection of chemotherapeutic agents influences the efficacy of embolization. The most appropriate chemotherapeutic agent for TACE has not been established.[10]

The aim of this study was to compare the efficacy of CDDP and EPI in TACE for patients with unresectable or relapsed HCC. We focused the number of tumors and the efficacy was adjusted for the confounding factors defined in this study.

303

METHODS

THE KYOTO UNIVERSITY Graduate School and Faculty of Medicine Ethics Committee approved this study in accordance with ethics guidelines for epidemiological studies in Japan (E-692). All patients gave informed consent for TACE.

Study design and eligibility criteria

We performed a historical cohort study to compare the efficacy of the first TACE with CDDP (CDDP-TACE) with that of the first TACE with EPI (EPI-TACE). Between 1 January 2003 and 31 December 2008, patients at Kyoto University Hospital who were diagnosed with HCC and treated with TACE were selected for this study. All patients were followed until 31 March 2009. In this study, we defined first TACE as TACE either for previously untreated HCC or for HCC that relapsed after curative hepatectomy, radiofrequency ablation (RFA) or percutaneous ethanol injection therapy (PEIT). The eligibility criteria of this study were the first TACE with or without simultaneous RFA or PEIT as a supplementary treatment. Patients under treatment for another type of cancer and/or who were diagnosed with extrahepatic metastasis of HCC were excluded. Patients who had been treated with hepatic arterial infusion chemotherapy and/or transcatheter arterial injection were also excluded.

Treatment

Using Seldinger's method, either powdered CDDP suspended in lipiodol or EPI dissolved in a contrast medium suspended in lipiodol was injected as selectively as possible into the hepatic segmental artery supplying the HCC. We had not selected a chemotherapeutic agent at random. EPI is commonly selected in clinical practices. CDDP was selected mainly because of a clinical research trial for the safety of TACE with CDDP.[11] Dosage of a chemotherapeutic agent was decided by the tumor load of HCC and it was administrated as 4 mg EPI and 10–20 mg CDDP per 1 cm of tumor diameter. The feeding arteries were embolized using a gelatin sponge.[12] A repeat TACE was not scheduled without a relapse of the tumors or insufficient treatment.

Assessment of efficacy

The efficacy of TACE was assessed by dynamic computed tomography (CT) or magnetic resonance imaging (MRI) scan within 3 months of first TACE. At least two medical doctors specializing in surgery or radiology at Kyoto University Hospital evaluated all CT or MRI scans.

Response to treatment was defined according to the European Association for the Study of the Liver (EASL) criteria.[13] The efficacy of TACE was evaluated as the response rate (RR), that is, the proportion of patients showing a complete or partial response.

Overall survival (OS) was defined as the interval between the day of the first TACE and the day of death from any cause. Patients who failed to undergo follow-up procedures were excluded on the last day when they were confirmed to be alive.

Confounding factors

Extraneous factors that are related to both the intervention and the outcome may distort the relationship in a study.[14] These extraneous factors are known as confounding factors.[14]

In HCC, prognostic factors such as the number of tumors, tumor diameter and vascular invasion have been established and some staging systems are constructed based on the prognostic factors, tumor markers and Child–Pugh classification.[15,16] TACE is selected according to algorithms that are decided by either prognostic factors or the staging system.[15] The selection criteria of TACE are practically changed by some factors such as previous hepatectomy, location of recurrent HCC and vascular invasion.

We defined the number of tumors, tumor diameter, vascular invasion, α-fetoprotein (AFP), prothrombin induced by vitamin K absence or antagonist-II (PIVKA-II), history of hepatectomy and RFA or PEIT combined with TACE as confounding factors.

We included RFA or PEIT combined with TACE in the eligibility criteria because it can be practically done after TACE whichever chemotherapeutic agent is selected. Furthermore, we defined it as a confounding factor. However, it would affect the RR directly. Therefore, we additionally estimated the RR in the patients without RFA or PEIT combined with TACE.

Statistical analyses

JMP for Windows software ver. 8.0 was used for all statistical analyses. We used the χ^2-test (without Yates' correction) for categorical comparisons of patient characteristics and confounding factors. Student's *t*-test was used to detect differences in the means of continuous variables. Probability of survival was calculated by the Kaplan–Meier method and examined using the logrank test. A *P*-value of less than 0.05 indicated statistical significance. All tests were two-tailed.

We calculated the relative risk for the RR and the associated 95% confidence interval (CI). In unadjusted analyses, we included only the chemotherapeutic agent as a covariate when calculating the odds ratio of the RR and the associated 95% CI within a logistic regression model. In adjusted analyses, we included all of the confounding factors in addition to the chemotherapeutic agent as covariates to calculate the odds ratio.

To test the interaction between the number of tumors and the chemotherapeutic agent, the product term that was constructed by the number of tumors and the chemotherapeutic agent was added as a covariate into the logistic regression model. After the test for interaction, we stratified patients by the number of tumors and estimated the odds ratio of the RR in both the unadjusted and adjusted analyses.

RESULTS

Patients

AMONG 940 CONSECUTIVE patients who were treated with TACE for HCC, 135 met the eligibility criteria. Four patients treated with EPI-TACE did not undergo any radiological imaging after the first TACE. Thus, 131 patients were enrolled in this study.

In total, 99 patients were treated with EPI-TACE and 32 patients were treated with CDDP-TACE. Median observation periods were 19 and 25 months for patients treated with CDDP-TACE and EPI-TACE, respectively. Patient characteristics are shown in Table 1. There were no statistical differences between the groups.

Relationship between RR and OS

We compared OS between responders and non-responders to the first TACE to examine the relationship between the RR and OS. The OS of responders to the first TACE was significantly longer than that of non-responders ($P = 0.0025$).

Response rates of patients treated with CDDP-TACE or EPI-TACE

The RR were 62.5% (20/32) and 51.5% (51/99) for patients treated with CDDP-TACE and EPI-TACE, respectively. In the unadjusted analysis, the relative risk and the odds ratio of CDDP-TACE versus EPI-TACE were 1.21 (95% CI = 0.87–1.69) and 1.56 (95% CI = 0.70–3.63), respectively. In the adjusted analysis, the odds ratio was 1.72 (95% CI = 0.70–4.48).

Test for interaction and stratification of patients by number of tumors

The test for interaction between the number of tumors and the chemotherapeutic agent was statistically significant in the adjusted analysis ($P = 0.016$). We stratified patients by the number of tumors and compared the efficacy. Both single HCC and multiple HCC groups were adjusted for the confounding factors (Table 2).

The RR of patients with a single tumor were 58.8% (10/17) and 65.2% (30/46) for CDDP-TACE and EPI-TACE, respectively. In the unadjusted analysis, the relative risk and the odds ratio of CDDP-TACE versus EPI-TACE for a single tumor were 0.90 (95% CI = 0.57–1.41) and 0.76 (95% CI = 0.024–2.45), respectively. In the adjusted analysis, the odds ratio for a single tumor was 0.43 (95% CI = 0.094–1.94).

On the other hand, the RR of patients with multiple tumors were 66.7% (10/15) and 39.6% (21/53) for CDDP-TACE and EPI-TACE, respectively. In the unadjusted analysis, the relative risk and the odds ratio of CDDP-TACE versus EPI-TACE for multiple tumors were 1.68 (95% CI = 1.03–2.74) and 3.05 (95% CI = 0.94–11.0), respectively. The odds ratio for multiple tumors was 4.11 (95% CI = 1.14–17.2) in the adjusted analysis (Table 3).

When the patients receiving the RFA or PEIT combined with TACE were excluded, RR of patients with a single tumor were 75.0% (9/12) and 65.3% (21/32) for CDDP-TACE and EPI-TACE and the RR of patients with multiple tumors were 71.4% (10/14) and 37.0% (17/46) for CDDP-TACE and EPI-TACE, respectively. For the patients with multiple tumors, the relative risk and the odds ratio were 1.93 (95% CI = 1.17–3.19) and 4.53 (95% CI = 1.22–16.8). CDDP-TACE also showed higher RR than EPI-TACE in this analysis.

DISCUSSION

THERE HAVE BEEN few randomized controlled studies comparing the effectiveness of different chemotherapeutic agents in TACE.[10] Four observational studies compared the efficacies of CDDP and doxorubicin in TACE. Three of these studies reported that TACE with CDDP showed a better survival rate than TACE with doxorubicin.[12,17,18] The fourth study did not find a significant difference between the two agents.[19] The present study could not demonstrate a clear difference in efficacy between CDDP-TACE and EPI-TACE. Nevertheless, the test for interaction between the number of tumors and the chemotherapeutic agent was statistically

Table 1 Characteristics of patients treated with EPI-TACE or CDDP-TACE

Patient characteristics	EPI-TACE ($n = 99$)	CDDP-TACE ($n = 32$)	P-value
Age			
Mean ± SD	68.4 ± 7.9	70.0 ± 5.3	0.29
Sex (%)			
Male	79 (80%)	25 (78%)	
Female	20 (20%)	7 (22%)	0.84
Hepatitis virus (%)			
HBsAg positive	17 (17%)	3 (9%)	
HCVAb positive	58 (59%)	22 (69%)	
Both positive	2 (2%)	1 (3%)	
Both negative	22 (22%)	6 (19%)	0.65
Child–Pugh classification (%)			
A	77 (78%)	27 (84%)	
B	22 (22%)	5 (16%)	0.42
PLT (%)			
<10^4/μL	33 (33%)	14 (44%)	
≥10^4/μL	66 (67%)	18 (56%)	0.29
Number of tumors (%)			
Single	46 (46%)	17 (53%)	
Multiple	53 (54%)	15 (47%)	0.48
Tumor diameter (%)			
<2 cm	66 (67%)	18 (56%)	
≥2 cm	33 (33%)	14 (44%)	0.29
Vascular invasion (%)			
Present	2 (2%)	2 (6%)	
Absent	97 (98%)	30 (94%)	0.23
AFP (%)			
<400 ng/mL	80 (82%)	27 (84%)	
≥400 ng/mL	18 (18%)	5 (16%)	0.72
PIVKA-II (%)			
<40 mAU/mL	47 (48%)	15 (48%)	
≥40 mAU/mL	52 (52%)	16 (52%)	0.93
History of hepatectomy (%)			
Present	68 (69%)	23 (71%)	
Absent	31 (31%)	9 (29%)	0.73
RFA or PEIT combined with TACE (%)			
Present	21 (21%)	6 (19%)	
Absent	78 (79%)	26 (81%)	0.76

AFP was not evaluated for one patient treated with EPI-TACE. PIVKA-II was not evaluated for another patient treated with CDDP-TACE. AFP, α-fetoprotein; CDDP, cisplatin; EPI, epirubicin; RFA, radiofrequency ablation; PIVKA-II, prothrombin induced by vitamin K absence or antagonist-II; PEIT, percutaneous ethanol injection therapy; TACE, transcatheter arterial chemoembolization; CDDP-TACE, the first TACE with CDDP; EPI-TACE, the first TACE with EPI; HBsAg, hepatitis B surface antigen; HCVAb, hepatitis C virus antibodies; PLT, platelets; SD, standard deviation.

significant. Therefore, we stratified patients by the number of tumors to compare the efficacy between CDDP-TACE and EPI-TACE.[20]

Previous studies reported that RR after TACE are similar to those obtained by mechanical occlusion alone.[10] Ischemia resulting from embolization may be the main factor inducing reduction in tumor size after TACE.[7] However, in this study, a difference in efficacy between the chemotherapeutic agents was observed in multiple HCC. Therefore, CDDP may have an additive antitumoral effect in multiple HCC. A previous study reported that CDDP appeared to yield the best RR when the antitumor efficacies of different anticancer agents were compared after intra-arterial infusion.[21] In TACE,

Table 2 Confounding factors of patients treated with EPI-TACE or CDDP-TACE after stratification of the number of tumors

Confounding factors	Single tumor			Multiple tumors		
	EPI-TACE ($n = 46$)	CDDP-TACE ($n = 17$)	P-value	EPI-TACE ($n = 53$)	CDDP-TACE ($n = 15$)	P-value
Tumor diameter (%)						
<2 cm	32 (70%)	13 (76%)		34 (64%)	5 (33%)	
≥2 cm	14 (30%)	4 (24%)	0.59	19 (36%)	10 (64%)	0.033
Vascular invasion (%)						
Present	0 (0%)	1 (6%)		2 (4%)	1 (7%)	
Absent	46 (100%)	16 (94%)	0.10	51 (96%)	14 (93%)	0.63
AFP (%)						
<400 ng/mL	37 (80%)	15 (88%)		43 (83%)	12 (80%)	
≥400 ng/mL	9 (20%)	2 (12%)	0.47	9 (17%)	3 (20%)	0.81
PIVKA-II (%)						
<40 mAU/mL	23 (50%)	10 (63%)		24 (44%)	5 (33%)	
≥40 mAU/mL	23 (50%)	6 (37%)	0.39	29 (56%)	10 (67%)	0.41
History of hepatectomy (%)						
Present	30 (65%)	14 (82%)		38 (72%)	9 (60%)	
Absent	16 (35%)	3 (18%)	0.19	15 (28%)	6 (40%)	0.39
RFA or PEIT combined with TACE (%)						
Present	14 (30%)	5 (29%)		7 (13%)	1 (7%)	
Absent	32 (70%)	12 (71%)	0.47	46 (87%)	14 (93%)	0.49

AFP was not evaluated for one patient with multiple tumors treated with EPI-TACE. PIVKA-II was not evaluated for another patient with single tumors treated with CDDP-TACE.

AFP, α-fetoprotein; CDDP, cisplatin; EPI, epirubicin; RFA, radiofrequency ablation; PIVKA-II, prothrombin induced by vitamin K absence or antagonist-II; PEIT, percutaneous ethanol injection therapy; TACE, transcatheter arterial chemoembolization; CDDP-TACE, the first TACE with CDDP; EPI-TACE, the first TACE with EPI.

Table 3 Risk ratio and odds ratio after stratification of the number of tumors

	Relative risk	95% CI	Odds ratio	95% CI
Single tumor				
Unadjusted	0.90	0.57–1.41	0.76	0.024–2.45
Adjusted			0.43	0.094–1.94
Multiple tumors				
Unadjusted	1.68	1.03–2.74	3.05	0.94–11.0
Adjusted			4.11	1.14–17.2

The unadjusted odds ratio of CDDP-TACE vs EPI-TACE is assessed within a logistic regression model that includes only the chemotherapeutic agent. The adjusted odds ratio is assessed within the logistic regression model that includes the chemotherapeutic agent and the confounding factors. CDDP-TACE, the first transcatheter arterial chemoembolization with cisplatin; CI, confidence interval; EPI-TACE, the first transcatheter arterial chemoembolization with epirubicin.

CDDP is gradually released into the systemic circulation.[22] CDDP might have effects on multiple HCC that include intrahepatic metastasis and tumors that are proliferating outside the capsule. Furthermore, CDDP is reported to be highly sensitive to some types of HCC.[23] The number of tumors may also be considered as a predictive factor for the sensitivity of CDDP.

On the other hand, CDDP-TACE may be less effective than EPI-TACE for single HCC. However, we practically use EPI in TACE for both simple and multiple HCC. Consequently, we are currently conducting a randomized controlled study to clarify the efficacies between CDDP and EPI in multiple HCC.

TACE is repeatedly used to treat relapses of HCC, as well as various therapeutic and prognostic factors influence the OS. On the other hand, the RR has been identified as an independent predictor of survival in HCC patients.[3,6,10] In this study, the OS in responders to the first TACE was significantly better than that in non-responders. Therefore, in this study, RR is considered to be more useful than OS for the evaluation of the efficacy of the chemotherapeutic agent in TACE. In this study, the 2-year survival rates were 84.5% for CDDP-TACE and 75.5% for EPI-TACE. For multiple HCC, the 2-year survival rates of patients were 77.0% for CDDP-TACE and 74.5% for EPI-TACE. TACE were repeated several times after the first TACE in most patients. Furthermore, repeated TACE with different chemotherapeutic agents were performed in 23 of 32 patients with CDDP-TACE and 43 of 99 patients with EPI-TACE.

Caution should be exercised in the use of CDDP, because CDDP has disadvantages such as the risk of nausea, vomiting, fever and thrombocytopenia.[11] Drug delivery systems (such as doxorubicin-loaded DEB) that promote long-lasting intratumoral retention of the drug may be means of decreasing systemic side-effects while increasing local chemotherapeutic effects.[8,9] The RR reported in a study investigating doxorubicin-loaded DEB was 66.6%,[8] which is similar to the RR in this study. Therefore, further studies will be needed to compare the contributions of drug delivery systems and/or chemotherapeutic agents to the efficacies of TACE.

In conclusion, the interaction between the number of tumors and the chemotherapeutic agent was observed in this study to compare the efficacy between CDDP-TACE and EPI-TACE. CDDP might be more effective than EPI in TACE for multiple HCC. A randomized controlled trial is needed to clarify the efficacy of CDDP, especially in patients with multiple HCC.

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Transcatheter arterial infusion chemotherapy with cisplatin–lipiodol suspension in patients with hepatocellular carcinoma

Masafumi Ikeda · Seishi Maeda · Hiroshi Ashihara ·
Hiroyasu Nagahama · Motohiko Tanaka ·
Yutaka Sasaki

Abstract

Purpose The aim of this study was to investigate the antitumor efficacy of treatment, identify prognostic factors, and construct a prognostic index in patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy (TAI) using cisplatin suspended in lipiodol. *Methods* We analyzed the outcomes in a total of 94 consecutive patients with previously untreated hepatocellular carcinoma who were treated by TAI using cisplatin suspended in lipiodol. *Results* Twenty-seven patients (29%) showed complete response and 21 patients (22%) showed partial response, with an overall response rate of 51% (95% confidence interval, 41–61%). The median survival time was 2.5 years and the proportions of survivors at 1, 2, and 5 years were 81.6, 65.2, and 18.3%, respectively. The results of multivariate analysis indicated a significant association of serum albumin ≥ 3.0 g/dL, maximum tumor size ≤ 3.0 cm, absence of ascites, and unilateral distribution of the tumors with a favorable survival. For clinical application, we also propose a prognostic index based on a combination of these prognostic factors. Based on this index, the patients were classified into three groups: those with good, intermediate, and poor prognosis. The median survival times in these three groups were 4.3, 2.7, and 1.1 years, respectively ($p < 0.01$). *Conclusions* TAI with cisplatin suspended in lipiodol exhibited favorable tumor efficacy and survival in patients with hepatocellular carcinoma. The prognostic factors identified and the index proposed based on these factors may be useful for predicting life expectancy, determining treatment strategies, and designing future clinical trials.

Keywords Hepatocellular carcinoma ·
Transcatheter arterial infusion chemotherapy ·
Cisplatin · Prognosis

Abbreviations

HCC	Hepatocellular carcinoma
TAE	Transcatheter arterial chemoembolization
TAI	Transcatheter arterial infusion chemotherapy
CT	Computed tomography
AFP	Serum alpha-fetoprotein
PIVKA II	Protein induced by vitamin K absence or antagonist-II
CR	Complete response
PR	Partial response

M. Ikeda · H. Ashihara · H. Nagahama · M. Tanaka ·
Y. Sasaki (✉)
Department of Gastroenterology and Hepatology,
Graduate School of Medical Sciences, Kumamoto University,
1-1-1, Honjo, Kumamoto 860-8556, Japan
e-mail: sasakiy@kumamoto-u.ac.jp

M. Ikeda
Division of Hepatobiliary and Pancreatic Oncology,
National Cancer Center Hospital East, Kashiwa, Chiba, Japan

S. Maeda
Department of Gastroenterology and Hepatology,
Tamana Regional Health Medical Center, Kumamoto, Japan

Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignancies in the world, and its incidence is continuing to increase worldwide. However, the prognosis of advanced HCC remains unsatisfactory [1]. Curative therapies such as resection, liver transplantation, and local ablative treatments may offer a chance of improved life

expectancy, but these treatment modalities are applicable to only a small proportion of all HCC patients. Transcatheter arterial chemoembolization (TAE) has been recognized as an effective palliative treatment option for patients with advanced HCC, because two meta-analyses [2, 3] of seven randomized controlled trials [4–10] showed that TAE significantly improves the survival of unresectable HCC patients with preserved hepatic function [1]. Transcatheter arterial infusion chemotherapy (TAI) is also often used for the treatment of advanced HCC, but a consensus regarding the most effective chemotherapeutic regimen has not yet been reached [11, 12]. Lipiodol, a lipid lymphographic agent, is selectively retained by HCC tissues for prolonged periods in comparison with non-cancerous tissues, and is therefore commonly mixed with anticancer agents to allow these agents to be retained for prolonged periods of time in the target tumor [13–15]. In a randomized controlled trial of TAE and TAI with zinostatin stimalamer and lipiodol, TAE did not yield superior survival as compared to TAI in patients with advanced unresectable HCC [16]. Our previous analysis also revealed that TAE did not significantly improve the survival of patients with HCC in comparison with TAI using cisplatin suspended in lipiodol, even though TAE is known to have higher antitumor efficacy than TAI [17]. Thus, TAI may have a higher efficacy on survival compared to TAE. If the appropriate indications for TAI can be expanded, additional embolization may not be necessary in some patients, considering that TAE has more deleterious effects on the liver functions than TAI [17, 18]. However, proper patient selection for TAI with lipiodol has not yet been fully investigated, although those for TAI without lipiodol [19–21] and for TAE [22–24] have been frequently analyzed. Analysis of prognostic factors would suggest appropriate patient selection for TAI. The present study was conducted to investigate the antitumor efficacy of the treatment, and to evaluate a number of variables that may affect survival in patients with HCC treated by TAI using cisplatin suspended in lipiodol; we have proposed a prognostic index in patients treated with TAI based on the results of our analyses.

Materials and methods

Patients

Between October 1987 and May 1996, 94 consecutive patients with previously untreated HCC were treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol at Kumamoto University Hospital, Japan. The study subjects were patients who were judged to

be suitable candidates for TAI (Table 1). HCC was diagnosed on the basis of histological examination or distinctive findings on computed tomography (CT) and/or angiography, associated with elevated serum levels of serum alpha-fetoprotein (AFP) or protein induced by vitamin K absence or antagonist-II (PIVKA II). Pretreatment evaluation included a complete medical history and careful physical examination. The laboratory procedures included complete

Table 1 Patient characteristics

	No of patients (%)
Host-related variables	
Age (years)	
Median [range]	64 [41–81]
Gender	
Male	62 (66%)
Blood transfusion	
Present	28 (30%)
Alcohol abuse[a]	
Present	11 (12%)
Smoking habit[b]	
Present	31 (33%)
Hepatitis B surface antigen	
Positive	14 (15%)
Hepatitis C antibody	
Positive	76 (81%)
Ascites	
Present	14 (15%)
Child-Pugh class	
A	45 (48%)
B	48 (51%)
C	1 (1%)
Tumor-related variables	
Number of tumors	
Multiple	53 (56%)
Tumor distribution	
Unilateral	70 (74%)
Maximum tumor size (cm)	
Median [range]	2.9 [1.5–12.0]
Portal vein invasion	
Present	7 (7%)
Alpha-fetoprotein (ng/mL)	
Median [range]	36.9 [1.9–17,100]
PIVKA II (mAU/mL)	
Median [range]	30 [0–6,000]
Other variables	
Modified Japan Integrated Stage	
Median [range]	2 [0–5]

PIVKA II protein induced by vitamin K absence or antagonist-II

[a] Ethanol intake ≥80 g/day for ≥5 years

[b] >20 cigarettes/day for >10 years

differential blood count, biochemistry tests, viral markers, including serum hepatitis B surface antigen and serum hepatitis C antibody, and tumor markers, including the serum levels of AFP and PIVKA II. Before treatment, a chest X-ray and ultrasonography and CT of the abdomen were obtained to evaluate the extent and size of the tumors and to exclude the presence of extrahepatic metastasis. The number, size, and distribution of the tumors were examined by CT and/or angiography. Written informed consent was obtained from all the patients prior to the start of the treatment.

Treatment procedure

Following conventional visceral angiography, TAI was performed by selectively introducing a catheter into the proper, right or left hepatic artery, or a branch of the artery feeding the tumor and injecting cisplatin suspended in lipiodol (iodized oil; Guerbet, Paris, France). The dose of the drug was determined based on the tumor size and liver function. The cisplatin suspension in lipiodol was prepared by the following procedure [25]: cisplatin powder, produced by evaporating water and sodium chloride from cisplatin solution, was sterilized by heating and subsequently suspended in lipiodol with a mortar and pestle under sterile conditions. The content of cisplatin in the lipiodol was adjusted to 20 mg/mL.

After the treatment, follow-up examinations, including CT, tumor marker measurement, and serum biochemistry, were performed, first at one month after the treatment completion and subsequently every 3–4 months. The transcatheter arterial treatments were repeated when relapse of the treated lesions and/or new hepatic lesions were seen.

Evaluation of the antitumor efficacy

The antitumor effect was assessed by contrast-enhanced CT or magnetic resonance imaging at one month after the treatment. Lipiodol accumulation in the tumor was regarded as representing necrotic tissue, because earlier studies have shown that areas on the CT showing lipiodol retention correspond to necrotic areas in the tumors [13–15]. We defined complete response (CR) as disappearance or 100% necrosis of all tumors, and partial response (PR) as >50% reduction and/or necrosis in the sum of all measurable tumors. Progressive disease was defined as more than 25% enlargement in the sum of all lesions and/or the appearance of any new lesions. Stable disease was considered as any disease that did not qualify for classification as CR, PR or progressive disease.

Factors analyzed

The relationships of pretreatment clinical variables to survival were investigated by univariate and multivariate analyses. The pretreatment variables were chosen based on their possible effects on the prognosis and tumor response indicated by previous investigations [1–12, 16–30] or suggested by our own clinical experience. Each of the variables, which were classified as host-related or tumor-related, was divided into two subgroups in accordance with clinically meaningful values for easy application in clinical practice, as shown in Table 2.

Overall survival was measured from the date of initial treatment to the date of death or last follow-up. Survival curves were calculated by the Kaplan–Meier method, and differences in survival were evaluated by the log rank test. The Cox proportional hazard model was used to determine the most significant variables related to survival. Forward and backward stepwise regression procedures based on the partial likelihood ratio were used to determine the major independent predictors of survival. A prognostic index based on the regression coefficients derived from all variables identified by the multivariate analysis was constructed. Stratification of the patients was conducted on the basis of this prognostic index. All p values presented in this report are of the two-tailed type. Differences at $p < 0.05$ were considered to be significant.

Results

Patient characteristics

The characteristics of all the 94 patients are shown in Table 1. There were 62 males (66%) and 32 females (34%), with a median age of 64 (range 41–81) years. There were 45 patients (48%), 48 patients (51%) and 1 patient (1%) with Child-Pugh stage A, B, and C [29], respectively. Fifty-three patients (56%) had multiple tumors, and the median maximum tumor size was 2.9 (range 1.5–12.0) cm. The median modified Japan Integrated Stage [30] was 2 (range 0–5). The median number of courses of TAI was two (range 1–9) during the follow-up period, and the median follow-up duration was 2.5 years (range 0.2–8.4 years). The median dose of cisplatin at first TAI was 50 (range 20–150) mg per treatment.

Treatment efficacy and survival

Twenty-seven patients (29%) showed CR and 21 patients (22%) showed PR, with an overall response rate of 51% (95% confidence interval, 41–61%). The median survival time was 2.5 years, and the proportions of survivors at 1, 2, 3, and 5 years were 81.6, 65.2, 39.8, and 18.3%, respectively (Fig. 1). The cause of death was tumor progression in 47 patients, hepatic failure in 25 patients, rupture of esophageal varices in 4 patients, and other causes in 6

Table 2 Univariate analysis of prognostic factors in patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol

	n	Median survival (years)	2-year survival (%)	Hazard ratio	p value
Host-related variables					
Age (years)					
≥60	67	2.5	65		
<60	27	2.6	54	0.98 (0.60–1.59)	0.93
Gender					
Female	32	2.7	66		
Male	62	2.4	60	0.99 (0.61–1.56)	0.97
Blood transfusion					
Present	28	2.5	60		
Absent	66	2.7	63	0.77 (0.48–1.24)	0.28
Alcohol abuse[a]					
Present	11	2.0	55		
Absent	83	2.6	63	0.63 (0.33–1.20)	0.16
Smoking habit[b]					
Absent	63	2.5	59		
Present	31	3.4	69	0.79 (0.50–1.27)	0.33
HBs Ag					
Negative	80	2.5	64		
Positive	14	1.8	46	0.77 (0.40–1.49)	0.45
HCV Ab					
Negative	18	1.9	47		
Positive	76	2.5	65	0.93 (0.53–1.64)	0.81
Ascites					
Present	14	1.4	21		
Absent	80	2.8	69	0.29 (0.16–0.53)	**<0.01**
WBC (×10^4/mm^3)					
≤4.0	51	2.5	61		
>4.0	43	2.5	64	0.76 (0.49–1.19)	0.23
Hemoglobin (g/dL)					
<10	17	2.4	59		
≥10	77	2.6	63	0.69 (0.40–1.19)	0.18
Platelet (×10^4/mm^3)					
<7.5	36	2.5	67		
≥7.5	58	2.5	59	0.89 (0.57–1.37)	0.59
Total bilirubin (mg/dL)					
≥2.0	13	1.8	46		
<2.0	81	2.7	65	0.59 (0.32–1.09)	0.09
Albumin (g/dL)					
<3.0	33	1.6	35		
≥3.0	61	4.0	76	0.29 (0.18–0.47)	**<0.01**
AST (U/L)					
≥85	24	2.4	58		
<85	70	2.8	63	0.63 (0.38–1.04)	0.07
ALT (U/L)					
≥92	21	2.4	57		

Table 2 continued

	n	Median survival (years)	2-year survival (%)	Hazard ratio	p value
<92	73	2.7	63	0.74 (0.44–1.24)	0.25
LDH (U/L)					
≥500	9	1.8	44		
<500	85	2.5	64	0.76 (0.36–1.58)	0.46
Prothrombin time (%)					
<70	41	2.4	58		
≥70	53	2.7	65	0.93 (0.60–1.45)	0.76
ICG R15 (%)					
≥30	46	2.2	52		
<30	43	3.4	71	0.68 (0.43–1.07)	0.09
Tumor-related variables					
Number of tumors					
Multiple	53	2.0	51		
Single	41	2.8	76	0.63 (0.41–0.98)	**<0.05**
Tumor distribution					
Bilateral	24	1.1	27		
Unilateral	70	2.8	73	0.39 (0.24–0.65)	**<0.01**
Maximum tumor size (cm)					
>3.0	40	1.6	42		
≤3.0	54	3.2	76	0.41 (0.26–0.66)	**<0.01**
Portal vein invasion					
Present	7	1.0	17		
Absent	87	2.6	65	0.36 (0.15–0.84)	**<0.05**
Alpha-fetoprotein (ng/mL)					
≥100	46	2.4	57		
<100	48	2.6	67	0.66 (0.42–1.02)	0.06
PIVKA II (mAU/mL)					
≥100	14	1.1	34		
<100	80	2.7	67	0.53 (0.29–0.97)	**<0.05**

p values lesser than 0.05 are given in bold

HBs Ag hepatitis B surface antigen, *HCV Ab* hepatitis C antibody, *WBC* white blood cell count, *AST* aspartate aminotransferase, *ALT* alanine aminotransferase, *LDH* lactic dehydrogenase, *ICG* indocyanine green test, *PIVKA II* protein induced by vitamin K absence or antagonist-II

[a] Ethanol intake ≥80 g/day for ≥5 years

[b] >20 cigarettes/day for >10 years

patients. Neither severe toxicity including renal dysfunction or thrombocytopenia, nor complication or treatment related death were seen in the present study.

Univariate and multivariate analysis

The median survival times, two-year survival, hazard ratios and p values of the survival time for univariate analysis are shown in Table 2. Among the host-related factors, absence of ascites and a serum albumin level of >3.0 g/dL were

significantly associated with a longer survival time. Among the tumor-related factors, single nodule, unilateral distribution of tumors, maximum tumor size <3.0 cm, absence of portal vein invasion, and PIVKA II level <100 mAU/mL were significantly associated with a longer survival time. The results of multivariate analysis using the Cox proportional hazard model are shown in Table 3. In the multivariate analyses, only those variables identified as significant by the univariate analysis were entered. Serum albumin ≥3.0 g/dL, maximum tumor size <3.0 cm, absence of ascites, and unilateral distribution of the tumors were significantly associated with favorable survival.

Risk groups based on the regression model

For the clinical application of these findings, a prognostic index was calculated based on the regression coefficients derived from the four variables identified by multivariate analysis (Table 3), as follows: prognostic index = score for albumin (0 for ≥3.0, 1 for <3.0 g/dL) + score for ascites (0 for absence, 1 for presence) + score for maximum tumor size (0 for ≤3.0, 1 for >3.0 cm) + score for tumor distribution (0 for unilateral, 1 for bilateral). The index values ranged from 0 to 4. The patients were then classified into three groups according to the prognostic index, as follows: good prognosis group (Group A: prognostic index = 0, $n = 31$ patients) (equivalent to patients with none of the four prognostic factors); intermediate

prognosis group (Group B: prognostic index = 1, $n = 28$ patients) (equivalent to patients with one of the four prognostic factors); poor prognosis group (Group C: prognostic index ≥ 2, $n = 35$ patients) (equivalent to patients with two or more of the four prognostic factors). The survival curves for the three groups are shown in Fig. 2. The median survival times in the good, intermediate, and poor prognosis groups were 4.3, 2.7, and 1.1 years, respectively. There were significant differences in the survival time among the three groups ($p < 0.01$).

Discussion

TAE has been widely used for cases with unresectable HCC and is currently the mainstay of non-surgical treatment for HCC, because it has been shown to exert a marked antitumor effect against HCC and can be administered for any type of HCC, regardless of the size, location or number of tumors [1]. In addition, the survival benefit of this treatment modality has been verified by two meta-analyses [2, 3] of seven randomized controlled trials [4–10]. However, TAE has deleterious effects on liver functions, thereby impairing the baseline prognosis. On the other hand, TAI has milder hepatotoxicity, but also shows a lower antitumor efficacy against advanced HCC than TAE. However, in a randomized controlled trial of TAE versus TAI with zinostatin-stimalamer and lipiodol, TAI and TAE were reported to yield comparable survival [16]. Moreover, the result of our retrospective analysis of TAE versus TAI using cisplatin–lipiodol suspension indicated similar outcomes for the two modalities [17]. From the results of these two studies, we could not conclude that additional embolization is not necessary for the treatment of advanced HCC, but there may be a subset of patients of advanced HCC in which TAI alone may yield sufficient treatment efficacy and survival. Therefore, this analysis of prognostic factors was carried out to enable identification of appropriate candidates for TAI using cisplatin–lipiodol suspension among HCC patients with no prior treatment. This single-institution study was undertaken using a unified method for tumor staging and identical procedures for treatment, follow-up, and supportive care throughout the duration of the study, to enable us to obtain reliable results for confirming important
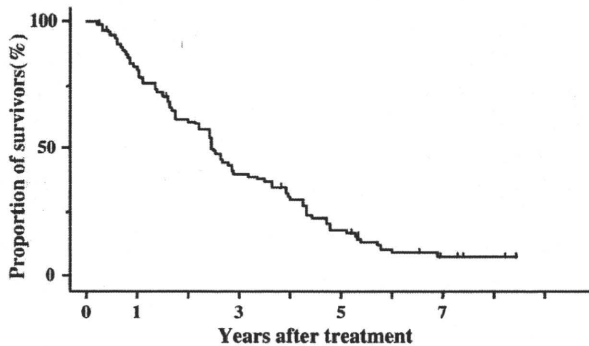

Fig. 1 Overall survival curve for all patients with hepatocellular carcinoma treated by transcatheter arterial infusion chemotherapy using cisplatin suspended in lipiodol. *Tick marks* indicate censored cases

Table 3 Significant prognostic factors determined by multivariate analysis with the Cox proportional hazard model

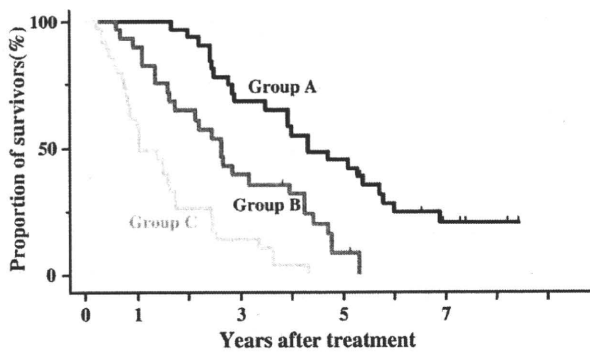| Variable	Coefficient	Hazard ratio (95% confidence intervals)	p value
Albumin ≥3.0 g/dL	0.94	0.39 (0.23–0.66)	<0.001
Maximum tumor size ≤3.0 cm	1.01	0.37 (0.19–0.69)	0.001
Absence of ascites	0.81	0.45 (0.11–0.40)	0.002
Unilateral tumor distribution	0.77	0.46 (0.27–0.79)	0.004

Fig. 2 Survival curves for the three groups determined by a prognostic index. *Group A* good prognosis (31 patients), *Group B* intermediate prognosis (28 patients), *Group C* poor prognosis (35 patients). *Tick marks* indicate censored cases

prognostic factors, predicting life expectancy and designing future clinical trials of TAI for HCC.

In this study, cisplatin was administered as the anticancer agent for TAI. Cisplatin has been reported to exert its actions by binding to the DNA in cancer cells, inhibiting DNA synthesis and subsequent cellular division. It is one of the key drugs for advanced HCC, that constituted a component of the combined chemotherapeutic regimen used in three of the seven randomized controlled trials of TAE reported until date [6, 7, 9]. In Japan, a favorable tumor response (33.8%) was reported in a clinical study of intra-arterial administration of cisplatin for advanced HCC [21], and the treatment has been approved for the treatment of HCC by the Ministry of Health, Labour and Welfare of Japan. Lipiodol has been used as a carrier for anticancer agents in targeting chemotherapy [13–15], and a suspension of cisplatin powder in lipiodol was used in this study. It has been reported that stronger antitumor effect is obtained by hepatic arterial administration of a combination of lipiodol and an anticancer agent than by that of an anticancer agent alone [26]. Recently, a lipophilic cisplatin derivative that can be suspended in lipiodol, SM-11355, was reported to show promising tumor efficacy (CR rate: 56%) in a phase II trial, and further trial is ongoing [27]. Therefore, combined therapy with cisplatin and lipiodol has been expected to become established as a valid option for the treatment of HCC. The response rate (51%: 95% confidence interval, 41–61%) at one month obtained in this study was more favorable than that in a clinical study of cisplatin alone, because TAI with an emulsion of an anticancer agent and lipiodol could be expected to exert more potent effects than an anticancer agent alone. However, follow-up at one month might be insufficient for evaluation of the rate/pattern of recurrence of HCC.

The median survival time and survival rates at two years in the current study were 2.5 years and 65.2%, respectively. These results were comparable or superior to those

of TAE reported from the aforementioned seven randomized controlled trials [4–10]. Although the study was based on a retrospective cohort design, the treatment efficacy of TAI with cisplatin–lipiodol suspension was promising and comparable to that of TAE for HCC.

In regard to the host-related factors, absence of ascites and a serum albumin level >3.0 g/dL were found to be favorable prognostic factors by multivariate analysis. Ascites and albumin are the most important factors to consider when evaluating the hepatic reserve, being included in both the Okuda staging system [28] and Child-Pugh classification [29], and have been shown to be prognostic factor in previous studies of patients with advanced HCC [19, 20, 22–24]. In regard to the tumor-related factors, a maximum tumor size ≤3.0 cm and unilateral distribution of the tumors were identified as being significantly associated with a longer survival time by multivariate analysis. Increased tumor size and bilateral distribution of tumors are the well-known unfavorable prognostic factors in HCC patients, and have been shown to be correlated with increased tumor volume and poorer differentiation of HCC, which reflect a more advanced stage and higher malignant potential of the tumors [22]. However, these prognostic factors for TAI with lipiodol in this study were similar to those identified for TAI without lipiodol [19–21] or TAE in previous reports [22–24], and no specific prognostic factors for TAI could be identified in this study.

For clinical application of these findings, we propose a prognostic index based on the independent prognostic factors identified in this study. Patients could be classified into three groups: those with good, intermediate, and poor prognosis ($p < 0.0001$) (Fig. 2). This index consists of both hepatic reserve and tumor stage, like the modified JIS score [30], and it differs from the Child-Pugh stage or TNM stage which are, respectively, based on either only the hepatic reserve or tumor stage. An index based on both the hepatic reserve and tumor stage might enable a more accurate prediction of life expectancy and stratification of the group into more distinct prognoses. This index can be easily calculated, because it is based on variables obtained during routine examinations before TAI. It can, therefore, be used to stratify patients with HCC before TAI according to the predicted survival. Accordingly, patients with good prognosis may obtain sufficient treatment efficacy and survival with TAI alone. In contrast, patients with a poor prognosis may be treated with supportive care only because of the extremely short median survival (1.1 years) expected, or may be treated other more aggressive treatments, such as more intensive chemotherapy. Recently, systemic chemotherapy for advanced HCC has become an important treatment modality, because sorafenib has been proven to confer a survival benefit and to show promise as a standard

treatment for patients with advanced HCC [31]. To improve the treatment efficacy, further chemotherapy regimens, such as the combination therapy comprising TAI with cisplatin suspended in lipiodol and sorafenib or other molecularly targeted agents, remain as challenges to be met following further detailed investigations. These findings may be helpful in predicting the life expectancy in HCC patients treated with TAI and provide more information to stratify patients in future TAI trials. It is also important to validate this prognostic index by applying it to other populations of HCC patients.

In conclusion, TAI with cisplatin suspended in lipiodol exhibited favorable tumor efficacy and survival in patients with HCC. Although no specific prognostic factors for TAI could be identified in this study, the results of the prognostic factors and the prognostic index may be helpful for predicting life expectancy, determining the most appropriate treatment strategies, and designing future clinical trials.

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Chronic hepatitis C viral infection reduces NK cell frequency and suppresses cytokine secretion: Reversion by anti-viral treatment

Omar Dessouki[a], Yasuyuki Kamiya[b], Hiroyasu Nagahama[b], Motohiko Tanaka[b], Shinya Suzu[a], Yutaka Sasaki[b], Seiji Okada[a,*]

[a] Division of Hematopoiesis, Center for AIDS Research, Kumamoto University, Kumamoto, Japan
[b] Department of Gastroenterology and Hepatology, Graduate School of Medical Sciences, Kumamoto University, Kumamoto, Japan

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ABSTRACT

Impaired activity of NK (natural killer) cells has been proposed as a mechanism contributing to viral persistence and chronic infection in hepatitis C (HCV) infection. We aimed to assess the impact of HCV infection on NK cells regarding frequency, subset distribution, and cytotoxic and cytokine secretion functions, as well as IFN-α and ribavirin therapeutic effects on NK cells. Significant reduction of total NK frequency and the CD56^{dim}16$^+$ subset was observed in chronic HCV patients. IFN-γ expression upon stimulation with K562 was severely suppressed but cytotoxicity measured by CD107a expression was maintained. These adverse effects were reversed after treatment with pegylated IFN-α and ribavirin; however, these skewed functions were not recovered in treatment-resistant patients. Thus, HCV chronic infection severely affects NK functions, except for cytotoxicity. Altered NK cell frequency and cytokine secretion by HCV infection may contribute to impaired cellular immune response and virus persistence.

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Introduction

Hepatitis C virus (HCV) infects about 3% of the global population, and it is estimated that only 20% of newly infected individuals will develop sufficient immune response to clear the infection [1]. Chronic infection with HCV results in degenerative liver disease that might end in cirrhosis or hepatocellular carcinoma [2]. Natural killer (NK) cells are considered to be the first line of defense against viral infections and cancer through their rapid cytotoxic activity and cytokine production [3]. Several controversial studies have reported the interaction between NK cells and HCV. These effects include suppression of HCV viral replication by NK cells, downregulation of NK natural cytotoxicity receptors by HCV, and cross-linking of HCV-E2 protein to the tetraspanin CD81 [4–7]. Significant numbers of NK cells are known to reside in the liver and to interact with virus infected hepatocytes [8,9]. A recent report showed that NK cells are polarized towards cytotoxicity during HCV infection, which could play a direct role in liver injury and the persistence

of HCV infection [10]. These studies emphasize the important role played by NK cells against HCV infection, as well as the immune evasion mechanisms implemented by HCV.

Combination therapy of pegylated IFN-α and ribavirin is now becoming the standard therapy against chronic HCV infection; however, the number of responding patients is limited to 50–70% [11,12], and the detailed molecular mechanisms of action of IFN-α are insufficiently understood. IFN-α anti-viral activity is not directly related to the virus or replication complex, but rather acts by inducing IFN-stimulated genes, which establish a non-virus-specific anti-viral state within the cell [13,14]. Enhancement of memory T-cell proliferation, prevention of T-cell apoptosis, stimulation of NK cell activation and dendritic cell maturation by Type 1 IFNs have been reported [15]. IFN-α has also complex and indirect effects on the immune system, which modify NK cell numbers and functions [7]. As NK cells are known to have strong anti-HCV activity [6,16], recovered NK cells have a key role in suppressing HCV replication after IFN-α treatment.

As most of the studies either assess treatment effect on NK cells in vitro, or compare different groups of patients, we aimed to assess the impact of HCV infection on NK cell activities through analyzing the same patients before and after treatment, excluding individual variations. We found that combination therapy of pegylated IFN-α and ribavirin reversed NK subtype distribution and functions in HCV-eliminated patients. Interestingly, relapsing patients failed to recover NK functions. These results suggest the