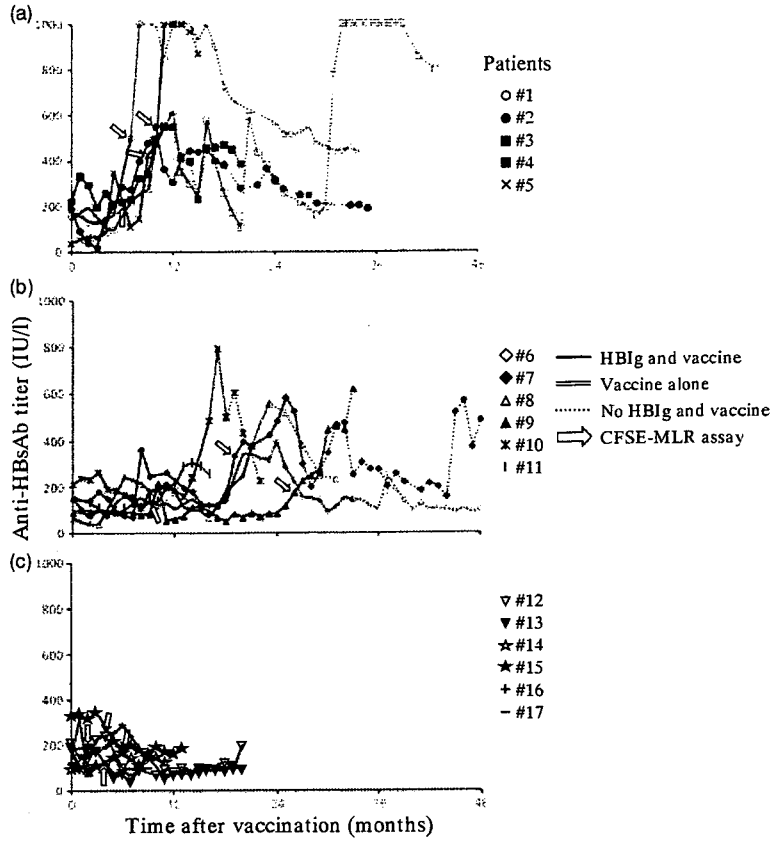

Figure 1 Anti-HBs titer kinetics in patients who responded to the vaccine within 1 year after commencement of vaccination (good responders) (a), in patients who responded to the vaccine after 1 year since the commencement of vaccination (moderate responders) (b), and in patients who did not respond to the vaccine (poor responders) (c).

Table 2. Age, gender, indication for LT, HBV viremia, immunosuppresive regimen, duration between vaccination and transplantation, and duration between steroid withdrawal and transplantation.

	Good responders (n = 5)	Moderate responders (n = 6)	Poor responders (n = 6)	P-value
Age at vaccination (years)*	55 (43–62)	46 (34–57)	48 (20–65)	NS
Gender (male/female)	5/0	4/2	4/2	NS
Indication for LT (fulminant hepatitis/cirrhosis)	1/4	1/5	1/5	NS
HBV DNA before LT (positive/negative)	2/3	2/4	2/4	NS
Recipient HBeAg before LT (positive/negative)	1/4	1/5	3/3	NS
Donor HBcAb before LT (positive/negative)	0/3	2/1	1/2	NS
Donor HBsAb before LT (positive/negative)	0/3	3/1	2/2	NS
CsA or Tac monotherapy/combination with steroid†	4/1	4/2	3/3	NS
Duration between vaccination and transplantation (months)*	24 (9–41)	21 (3–40)	17 (12–25)	NS
Duration between steroid withdrawal and transplantation (months)*	11 (1–45)	12 (1–50)	16 (1–29)	NS
Anti-HBsAb titer (IU/l)*†	152 (38–221)	139 (93–215)	191 (92–328)	NS

NS, not significant; LT, liver transplantation; CsA, cyclosporine A; Tac, tacrolimus.
*Median (range).
†At the time of vaccination.

MLR assay using a CFSE-labeling technique. In all the seven patients who responded to the HBV vaccine, limited $CD4^+$ T-cell proliferation was observed in the anti-donor MLR assay as compared with the anti-third-party MLR assay, i.e., a hyporesponse in the anti-donor MLR assay and a normal response in the anti-third-party MLR assay (Fig. 3). In these patients, the average of SIs for $CD4^+$ T cells in response to anti-third-party stimulation was >2 (average value in healthy volunteers without any immunosuppressive treatment). In contrast,

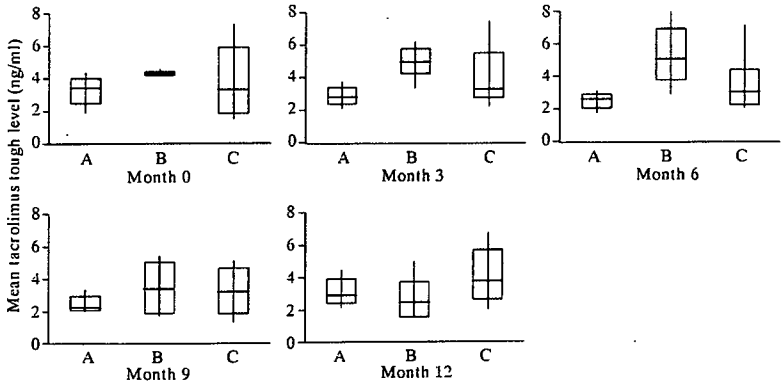

Figure 2 Tacrolimus trough levels in patients who responded to the vaccine within 1 year after commencement of vaccination (good responders) (A), in patients who responded to the vaccine after 1 year since the commencement of vaccination (moderate responders) (B), and in patients who did not respond to the vaccine (poor responders) (C). The Mann–Whitney *U*-test was used to compare the tacrolimus trough levels between the good and moderate responders with those of poor responders. The box plot represents the 25th to 75th percentile, the dark line is the median, and the extended bars represent the 10th to the 90th percentile. Statistical analyses at none of the time-points at 0, 3, 6, 9 and 12 months were significant.

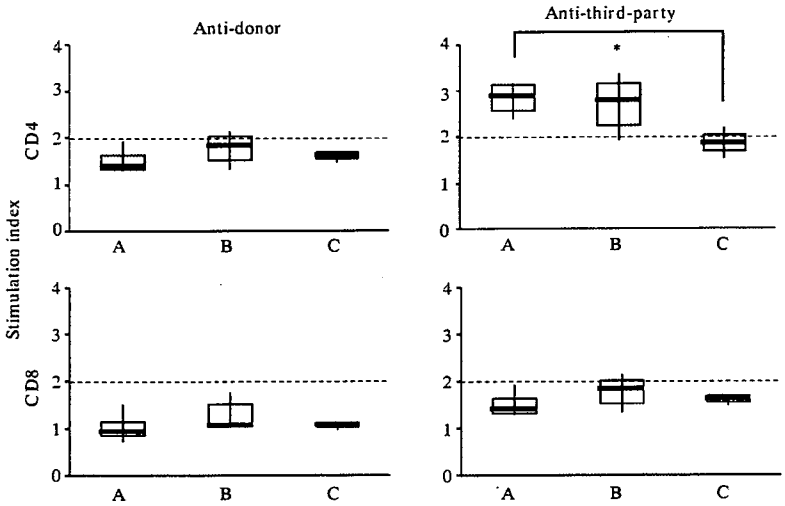

Figure 3 Stimulation indices (SIs) of each of the $CD4^+$ and $CD8^+$ T-cell subsets in the anti-donor and anti-third-party MLR in patients who responded to the vaccine within 1 year after commencement of vaccination (good responders) (A), in patients who responded to the vaccine after 1 year since the commencement of vaccination (moderate responders) (B), and in patients who did not respond to the vaccine (poor responders) (C). $CD4^+$ and $CD8^+$ T-cell proliferation and their SIs were quantified as follows. The number of division precursors was extrapolated from the number of daughter cells of each division, and the number of mitotic events in each of the $CD4^+$ and $CD8^+$ T-cell subsets was calculated. Using these values, the mitotic index was calculated by dividing the total number of mitotic events by the total number of precursors. The SIs of allogeneic combinations were calculated by dividing the mitotic index of a particular allogeneic combination by that of the self control. The Mann–Whitney *U*-test was used to compare the tacrolimus trough levels between the good and moderate responders with those of poor responders. The box plot represents the 25th to 75th percentile, the dark line is the median, and the extended bars represent the 10th to the 90th percentile. $^*P = 0.04$.

in the four patients who did not respond to the HBV vaccine, limited $CD4^+$ and $CD8^+$ T-cell proliferation was observed in both the anti-donor and the anti-third-party MLR assay, i.e., a hyporesponse in both cases. In these patients, the average of SIs for $CD4^+$ T cells in response to both anti-donor and anti-third-party stimulation was <2. Thus, the SIs for $CD4^+$ T cells in response to anti-third-party stimulation in good responders was higher than that of poor responders ($P = 0.04$) (Fig. 3).

Discussion

The strategy of HB vaccination after LT to achieve protective immunity and to allow discontinuation of long-term HBIg administration has been investigated in a number of studies [7,11,12,15–20]. However, those attempts to immunize these patients with HB vaccine have been equivocal and generally less than successful. It is common practice to immunize these patients against hepatitis B; however, the response of LT recipients could be below adequate standard. Although the currently available HBV vaccines are extremely safe and have an efficacy of more than 90% in the general population, it has been reported that the response rate is slightly lower in obese individuals, smokers, and men and is significantly lower in patients with cirrhosis or chronic renal failure, patients undergoing long-term hemodialysis, organ transplant recipients, and immunocompromised patients [21]. In particular, because of the impairment in T-cell-dependent functions in cirrhotic patients, the results of vaccination in transplant candidates have been very disappointing [25–29]. Moreover, even in responder patients, immunosuppressive treatment frequently leads to a decrease in the serum antibody titers after transplantation [21]. Among the previous HBV vaccination trials in multiple institutions, most of the results did not show significant promise with regard to HBV vaccine response rates. Each vaccination protocol differed with respect to the dose of vaccine, the time of commencement and frequency of vaccination, the route of vaccination, combination with HBIg, and the immunosuppressive regimen at the time of vaccination. It has been reported that successful vaccination is attributed to the long time-interval that had elapsed after transplant, which allowed them to markedly reduce the immunosuppressive therapy [11]. It has also been proposed that the administration of the vaccine through the intradermal route in preference to the intramuscular route might prove to be more responsive to HB vaccination, because the epidermis is known to be rich with antigen-presenting cells, making it an appropriate target for vaccine delivery [18]. Based on these hypotheses in this study, vaccination through the intradermal route was administered to the LT recipients against HBV with an effort to minimize immunosuppression. In addition to the different vaccination protocols, the difference in the immune status of the subjects likely influences their HBV vaccine response.

In order to evaluate the immune status of the LT recipient vaccinees, we employed a MLR assay using a CFSE-labeling technique [22]. CFSE stably stains intracellular proteins without toxicity, and the fluorescence of each stained cell segregates equally to the daughter cells upon cell division, resulting in sequential halving of cellular fluorescence intensity with each successive generation [30]. When analyzed by FCM, this sequential halving of fluorescence is visualized as distinct peaks or populations of cells and can be used to track cell division in populations of proliferating cells. This, then, allows phenotypic analysis of the proliferating cells and determination of the number of cells produced in each generation by multicolor FCM analysis, i.e., the number of viable $CD4^+$ and $CD8^+$ responder T cells that proliferate in response to allostimulation can be quantified separately. The lack of proliferation of $CD4^+$ T cells in anti-donor MLR reflects the suppression of the anti-donor response [22]. In this study, all of the good responders showed a normal response of the anti-third-party $CD4^+$ T cells (Fig. 3). In contrast, the poor responders showed a hyporesponse of both anti-donor and anti-third-party $CD4^+$ T cells, suggesting an excessively immunosuppressive state. The development of an effective immune response to HB vaccination requires coordinated immune activity comprising the interaction of T cells, cytokines, antigen-presenting cells, and B cells [31]. It is important to note that these immunocompetent cells can be sufficiently activated to acquire immune activity at the time of vaccination even in a state of immunosuppression. T-cell interaction should lead to (i) activation of anti-HBsAg-specific T cells in order to achieve a successful response to vaccination and (ii) suppression of anti-donor-specific T cells to avoid transplant rejection. Patients showing a donor-specific hyporesponse with a well-maintained response to the third-party stimulus always achieved a sustained immune response to the vaccine in this study; based on this observation, we propose a concept that inducing anti-donor-specific immunosuppressive status by minimizing immunosuppression enables post-transplant HBV vaccination to become a promising prophylactic strategy, although further studies are needed to establish the optimal HBV vaccination protocol. A larger and prospective trial might be required to evaluate whether or not the MLR response can actually predict successful HBV vaccination. The higher rate of response to vaccination than that of this study has been shown in a previous report [17]. An adjuvant preparation of vaccine that used in the previous study is thought to attribute to the successful induction of a strong response. It remains to elucidate whether patients with hyporesponse to both anti-donor and anti-third-party $CD4^+$ T cells can respond to such an adjuvant preparation of vaccine.

Authorship

HT, KC, and HO: designed research. HT and YT: performed research. HT, KI, KI, MS, TI, YU, MO, MB,

HT, TI, and TA: collected data. HT, YT, and HO: analyzed data. HT and HO: wrote the paper.

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Original Article

Chronic hepatitis C in patients co-infected with human immunodeficiency virus in Japan: a retrospective multicenter analysis

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Aim: A nationwide survey in Japan revealed that nearly one-fifth of human immunodeficiency virus (HIV)-positive patients are co-infected with hepatitis C virus (HCV). We conducted a study to further analyze the features of liver disease in HIV–HCV co-infected patients.

Methods: We analyzed 297 patients from eight hospitals belonging to the HIV/AIDS Network of Japan.

Results: HCV genotypes 1, 2, 3, 4 and mixed genotypes were detected in 55.2, 13.7, 18.9, 0.9 and 11.3% of patients, respectively, in contrast to the fact that only genotypes 1 and 2 are detected in HCV mono-infected patients in Japan. This is compatible with the transmission of HCV through imported blood products contaminated by HCV. Sixteen of 297 HIV–HCV co-infected patients had advanced liver disease accompanied by ascites, hepatic encephalopathy or hepatocellular carcinoma. The average age of such patients was 41.1 ± 14.0 years, which was much younger than that of HCV mono-infected patients with the same complications. The progression speed of liver disease estimated from the changes in the levels of serum albumin, bilirubin, or platelet was slower in patients who achieved sustained virological response with interferon treatment than in those who did not receive it. The overall sustained virological response rate to interferon treatment was 43.3%.

Conclusions: Our findings suggest that liver disease is more advanced in HIV–HCV co-infected patients than in HCV mono-infected patients, and interferon treatment may retard the progression of liver disease in such patients.

Key words: acquired immunodeficiency syndrome, chronic liver disease, genotype, interferon therapy

INTRODUCTION

THE PROGNOSIS OF human immunodeficiency virus (HIV) infection has markedly improved since the introduction of hyperactive anti-retroviral therapy (HAART).[1,2] Opportunistic infection has been prevented or properly managed, resulting in lower mortality rates. Liver disease, in particular related to hepatitis C virus (HCV) infection, has now become the main cause of mortality among HIV-infected patients on HAART in Western countries.[3,4] A national survey among Japanese HIV-infected patients with coagulation disorders has shown that the mortality rate related to HCV-related liver disease after 1997 was twofold that before 1997.[5] In Japan, therefore, HCV infection may also be a major cause of death in HIV–HCV co-infected patients. However, there has been no extensive analysis of liver disease in HIV–HCV co-infected patients in Japan.

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Interferon (IFN) treatment in combination with ribavirin administration, which is now the first choice for HCV mono-infected patients,[6] is also a standard treatment for chronic hepatitis in HIV–HCV co-infected patients. Eradication of HCV is assumed to improve liver function, and normalization of serum aminotransferase (ALT) levels by IFN treatment may retard the progression of liver disease in HIV–HCV co-infected patients, even if they are on HAART. However, in general, the response rate to IFN treatment is lower in HIV–HCV co-infected patients than in HCV mono-infected patients.[7] The effects of IFN treatment on liver function and prognosis in HIV–HCV co-infected patients in Japan are yet undefined.

In 2004, we conducted a nationwide survey to determine the prevalence of HCV infection in HIV-infected patients by distributing a questionnaire to the hospitals in the HIV/AIDS Network of Japan, which revealed that 935 (19.2%) of 4877 HIV-positive patients were also positive for anti-HCV antibody.[8] In this study, we analyzed the progression of liver diseases and the impact of IFN treatment on the parameters of liver function in HIV–HCV co-infected patients in a multicenter retrospective study.

METHODS

Registry of patients with HIV–HCV co-infection

THE QUESTIONNAIRE REGARDING the current state of HIV–HCV co-infection was sent to the 366 hospitals in the HIV/AIDS Network of Japan in 2004, sponsored by the Japanese Ministry of Health, Labour and Welfare. One hundred seventy-six hospitals (48.1%) responded. The results, already published,[8] showed that HIV–HCV co-infected patients are concentrated in particular hospitals in big cities around Japan. Among these hospitals, we chose three hospitals in the Tokyo metropolitan area, and one each in the Hokkaido, Chubu, Osaka, Chugoku and Kyushu areas. These eight hospitals belong to the HIV/AIDS Network and had more HIV–HCV co-infected patients than other hospitals.

In the study, the following information was obtained from the hospitals regarding each HIV–HCV co-infected patient who visited the hospitals at least once between January and December in 2004: (1) age and sex of HIV-positive patients with anti-HCV; (2) possible transmission routes of HIV; (3) history of habitual alcohol intake; (4) date of the first and last visits; (5) counts of white blood cells, CD4-positive lymphocytes and platelets at the first and last visits; (6) levels of serum albumin and bilirubin at the first and last visits; (7) levels of HIV-RNA and HCV-RNA at the first and last visits; (8) history of IFN treatment with or without ribavirin; (9) history of HAART; and (10) history of jaundice, ascites, hepatic encephalopathy and hepatocellular carcinoma (HCC). The study sheets were completed by the physicians in charge and sent to the Department of Internal Medicine, University of Tokyo.

Ethical issues

The protocol of the current survey was approved by the ethical committee of each institution, and written informed consent was obtained from each patient.

Statistical analysis

The collected data were analyzed using Mann–Whitney's *U*-test whenever appropriate. *P*-values less than 0.05 were regarded as statistically significant.

RESULTS

Clinical backgrounds of registered patients

FROM THE EIGHT hospitals, 297 patients were registered. The number, age, sex, estimated transmission routes and history of habitual alcohol intake are shown in Table 1. Two hundred and ninety (97.6%) were male patients. The mean age of the patients was 37.9 ± 10.3.

HCV genotype was determined in 212 patients. One hundred seventeen (55.2%) patients were infected by genotype 1 HCV. Infection by genotypes 2, 3 or 4 HCV was found in 29 (13.7%), 40 (18.9%) and 2 (0.9%) patients, respectively. Twenty-four (11.3%) patients were infected by HCV of mixed genotypes. In the remaining 85 patients, the genotype was indeterminable or undetermined. The mean ages of patients infected by different HCV genotypes were similar (Table 1).

In 259 (87.2%) of 297 registered patients, HIV was most probably transmitted through the administration of blood products. Other transmission routes were sexual contacts among men who have sex with men (MSM) (4.0%), heterosexual contacts (3.0%) and intravenous drug use (IDU) (0.3%). Habitual alcohol consumption was noted in only one patient with genotype 1 HCV (0.6%).

Outcomes of IFN treatment in HIV–HCV co-infected patients

Serum HCV-RNA levels were available both at the first visit and registry to the study (i.e. the end of observa-

Table 1 Demography, transmission route and HCV genotypes in HIV–HCV co-infected patients

HCV genotype	Number (%)	HCV sub-genotypes	Viral load† (High : Low)	Age	Sex (Male : Female)	Transmission route				
						Transfusion	MSM	Hetero-sexual	IDU	Others
1	117 (55.2)	1a 31, 1b 43, 1a+1b 31, undetermined 12	31:11	38.3 ± 10.4	114:3	102	7	1	0	7
2	29 (13.7)	2a 16, 2b 11, undetermined 2	5:5	39.8 ± 9.5	29:0	24	1	1	0	3
3	40 (18.9)	3a 40	12:2	36.1 ± 8.9	40:0	38	0	0	0	2
4	2 (0.9)	4a 2	2:0	38.5 ± 2.1	2:0	2	0	0	0	0
Mixed	24 (11.3)	2a+3a 6, 1b+3a 3, others 15	11:0	38.7 ± 8.7	24:0	24	0	0	0	0
Others	85	Undetermined 85	6:1	36.2 ± 11.5	81:4	69	4	7	1	4
Total	297		67:19	37.9 ± 10.3	290:7	259 (87.2%)	12 (4.0%)	9 (3.0%)	1 (0.3%)	16 (5.5%)

†Viral loads are available in only a subset of patients. High viral load: more than 1 Meq/mL by branched DNA-probe assay or more than 100 KIU/mL by Amplicor monitor assay.
HCV, hepatitis C virus; HIV, human immunodeficiency virus; IDU, intravenous drug users; MSM, men who have sex with men.

tion) in 158 patients. Of these 158, 60 patients (38.0%) received IFN treatment for HCV, and 35 of these 60 patients did it in combination with ribavirin. Those who did not complete the scheduled treatment were excluded from the current analysis.

As shown in Table 2, 26 (43.3%), 11 (18.4%) and 23 (38.3%) of the treated patients achieved sustained virological response (SVR), end-of-treatment virological response (ETR) and no virological response (NR), respectively. The SVR rate in patients with each genotype is shown in Table 2. The SVR rate in the patients who underwent IFN treatment in combination with ribavirin was 31.4% in total. The SVR rate in patients with each genotype who underwent IFN/ribavirin combination therapy is shown in Table 2.

All of the 26 patients who achieved SVR remained negative for serum HCV-RNA in the further follow-up periods. In contrast, none of the patients with ETR or NR became negative for serum HCV-RNA in the follow-up periods. In five patients who did not receive IFN treatment, HCV-RNA was negative at the end of the observation period, although it was positive at least twice before the registry. The profiles of the five patients are shown in Table 3.

Changes in liver function and associated complications (Table 4)

As mentioned above, the data on liver function and serum HCV-RNA positivity were available both at the first visit and registry (end of observation) in 158 of the 297 registered patients. The mean observation period was 9.5 ± 5.0 and 8.2 ± 8.2 years in the IFN-treated and IFN-untreated patients, respectively. Unfortunately, few, if any, patients underwent liver biopsy, because most HIV–HCV co-infected patients had coagulation disorders.

The annual change in the serum albumin concentration was +0.05 ± 0.42 g/dL in the IFN-treated patients, and 0.80 ± 0.82 g/dL in the non-IFN-treated patients. The annual change in the serum bilirubin concentration was +0.08 ± 0.38 mg/dL in the IFN-treated patients, while it was +0.15 ± 0.15 mg/dL in the non-IFN-treated patients. Among the IFN-treated patients, the serum bilirubin concentration decreased by 0.02 ± 0.08 mg/dL in the patients who achieved SVR, which was significantly larger than that in the non-IFN-treated patients at the end of the observation ($P < 0.05$). The annual changes in platelet counts were +0.06 ± 1.13 (10^4/ l) in the IFN-treated patients and 0.94 ± 0.95 (10^4/ l) in the non-IFN-treated patients. The change in platelet

Table 2 Virological response to interferon treatment in HIV–HCV co-infected patients

Genotype	Viral load (High : Low)†	Response			Total
		SVR	ETR	NR	
(a) Response to interferon treatment in total (with or without ribavirin)					
1	9:6	7 (33.3%)	1	13	21
2	5:3	4 (40.0%)	2	4	10
3	5:1	5 (62.5%)	1	2	8
4	1:0	0	1	0	1
Mixed	5:1	2 (33.3%)	3	1	6
Others	6:2	8 (57.1%)	3	3	14
Total	31:13	26 (43.4%)	11	23	60
(b) Response to ribavirin/interferon combination therapy including peginterferon					
1	8:2	2 (15.3%)	0	11	13
2	1:2	1 (25.0%)	0	3	4
3	4:1	4 (66.7%)	1	1	6
4	1:0	0	1	0	1
Mixed	4:1	1 (20.0%)	3	1	5
Others	3:0	3 (50.0%)	1	2	6
Total	21:6	11 (31.4%)	6	18	35

†Viral loads are available in only a subset of patients. High viral load: more than 1 Meq/mL by Branched DNA-probe assay or more than 100 KIU/mL by Amplicor monitor assay.
ETR, end of treatment virological response; NR, no virological response; SVR, sustained virological response.

counts in the patients who achieved SVR was significantly larger than that in the non-IFN-treated patients ($P < 0.05$, Table 4).

No symptoms of hepatic failure (ascites or hepatic encephalopathy) were observed in the 60 IFN-treated patients while they were observed in six of the 98 non-IFN-treated patients. HCC was found in one IFN-treated patient after SVR, while it was found in two non-IFN-treated patients (Table 4).

Impact of HAART on liver function and associated complications (Table 5)

Information on HAART was available in 292 patients. The mean observation periods were 8.4 ± 4.2 years in 234 patients on HAART, and 9.8 ± 6.0 years in 58 patients not on HAART. Changes in the levels of albumin, bilirubin or platelet were similar between the two groups (statistically not significant). The morbidities of hepatic decompensation symptoms (ascites and hepatic encephalopathy) and HCC were not significantly different between the two groups. In total, nine patients had hepatic decompensation and seven had HCC, and the average age of such patients was 41.1 ± 14.0 years, which was much younger than that of HCV mono-infected patients with the same complications.[9]

DISCUSSION

IN THE CURRENT study, the features of liver disease in HIV–HCV co-infected patients in Japan were analyzed. The determination of HCV genotypes revealed that genotype 3 or 4, which is rarely seen in HCV mono-infected patients in Japan,[10] was found in a substantial fraction of HIV-infected patients. In addition, some of these patients were infected with HCV of mixed genotypes. These results are compatible with the fact that HCV is transmitted through imported blood products that were contaminated by HCV, as is the case with HIV infection.[11] Infection by HCV of mixed genotypes may reflect frequent administrations of blood products of different lots.

We evaluated the response rate to IFN treatment in HIV–HCV co-infected patients in Japan. Because the IFN treatment protocol varied between facilities, it was not easy to evaluate the effects of the treatments including IFN in this cohort. However, the regimen of ribavirin/IFN combination therapy was similar between the hospitals: the treatment period was 24 weeks in patients with HCV genotypes 2 and 3, and 48 weeks in those with HCV of other genotypes when either pegylated or standard IFN in combination with ribavirin was used.[12] Therefore, it may be possible to estimate the effect

Table 3 Clinical backgrounds of patients who spontaneously cleared HCV in HIV-infected patients

Patient no.	Age	Sex	Transmission route	Observation period (years)	HCV-RNA (KIU/mL)	HCV genotype	HIV-RNA (10^2/mL)	WBC (/ L)	CD4+ T cells (/ L)	Platelets (10^4/mL)	ALT (U/l)	HAART
1	33	M	Transfusion	8.8	290	ND	200 000	4500	5	26.3	21	Yes
2	31	M	MSM	2.3	Positive†	ND	13 000	5760	931	22.7	29	Yes
3	27	M	Transfusion	9.3	>850	3a	180 000	4000	51	10.1	84	Yes
4	53	M	Transfusion	4.5	Positive†	1a	20 000	4800	296	35.4	24	No
5	22	M	Transfusion	7.8	220	ND	990	5500	125	33.1	44	Yes

†Positive: HCV-RNA was positive by qualitative PCR, but was not quantitatively determined.
ALT, aminotransferase; HAART, highly active anti-retroviral therapy; HCV, hepatitis C virus; HIV, human immunodeficiency virus; MSM, men who have sex with men; ND, not determined; WBC, white blood cells.

Table 4 Changes in clinical parameters and IFN treatment in HIV–HCV co-infected patients

	Outcome of IFN treatment	Number	Observation period (years)	⊿Albumin†	⊿Bilirubin‡	⊿Platelet§	Ascites/ encephalopathy	HCC
IFN-treated patients		60	9.5 ± 5.0	0.05 ± 0.42	0.08 ± 0.38*	0.06 ± 1.13	0	1
	SVR	26	9.1 ± 4.4	0.13 ± 0.59	( ) 0.02 ± 0.08*	0.14 ± 0.76*	0	1
	ETR	11	14.6 ± 7.0	( ) 0.07 ± 0.14	0.51 ± 1.04	0.07 ± 1.50	0	0
	NR	23	7.4 ± 2.0	0.01 ± 0.30	0.09 ± 0.30	( ) 0.18 ± 0.32	0	0
Non-IFN-treated patients		98	8.2 ± 8.2	( ) 0.80 ± 0.82	0.15 ± 0.15	( ) 0.94 ± 0.95	6	2
All		158	8.7 ± 4.7	( ) 0.45 ± 2.93	0.13 ± 0.52	( ) 0.59 ± 3.78	6	3

*$P < 0.05$ versus patients without IFN treatment.
†⊿Albumin: changes in albumin concentration (g/dL)/observation period (years).
‡⊿Bilirubin: changes in bilirubin concentration (mg/dL)/observation period (years).
§⊿Platelet: changes in platelet count (10^4/ L)/observation period (years).
ETR, end of treatment virological response; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus; IFN, interferon; NR, no virological response; SVR, sustained virological response.

Table 5 Changes in clinical parameters and HAART in HIV–HCV co-infected patients

	Number	Age	Sex (M : F)	Observation period (years)	⊿Albumin†	⊿Bilirubin‡	⊿Platelet§	IFN	Ascites/ encephalopathy	HCC
HAART (+)	234	37.8 ± 10.4	227:7	8.4 ± 4.2	( ) 0.002 ± 0.18	0.13 ± 0.53	( ) 0.40 ± 3.71	143 (61.1%)	6	5
HAART ( )	58	38.1 ± 10.5	58:0	9.8 ± 6.0	( ) 0.14 ± 0.18	0.03 ± 0.25	( ) 1.40 ± 3.30	30 (51.7%)	3	2

†⊿Albumin: changes in albumin concentration (g/dL)/observation period (years).
‡⊿Bilirubin: changes in bilirubin concentration (mg/dL)/observation period (years).
§⊿Platelet: changes in platelet count (10^4/[L)/observation period (years).
HAART, highly active anti-retroviral therapy; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; HIV, human immunodeficiency virus.

of ribavirin/IFN combination therapy in HIV–HCV co-infected patients in this study.

The response rate to ribavirin/IFN combination therapy was 31.4% in total, and 15.3% in patients with HCV genotype 1, which are comparable rates to those achieved in previous studies on HIV–HCV co-infected patients in Western countries.[7] The low response rate in HIV–HCV co-infected patients compared with HCV mono-infected patients[12] may be attributed to several factors: impaired immune response, high HCV loads and viral quasi-species caused by frequent chances of transmission. Of these, high viral loads may be essential, because Table 2 shows that patients with genotype 1 HCV achieved SVR even by IFN monotherapy if their viral loads were low. In the era of IFN monotherapy, patients with favorable conditions were treated first of all: pretreatment viral loads in patients who received IFN monotherapy were lower than those who received PEG-IFN–ribavirin combination therapy. This may be the reason why the efficacy of PEG-IFN–ribavirin combination therapy was lower than that with IFN monotherapy in this study.

The serum bilirubin concentrations and platelet counts were improved in the patients who achieved SVR by IFN treatment. Although the response rate to IFN treatment is lower in HIV–HCV co-infected patients than in HCV mono-infected patients, the overall benefit of IFN treatment on liver function may be similarly expected in the patients who achieved SVR. HAART showed no impact on the liver function in HIV–HCV co-infected patients. Improvement of liver function can be expected only in IFN-treated patients, although there is a possibility that only patients with preserved liver function were able to receive IFN treatment. Given that liver disease is the major life-threatening factor in HIV-infected patients, IFN treatment should be considered in the early stage of HIV–HCV co-infection.

It should be noted that nine patients had hepatic decompensation and seven had HCC, and the average age of such patients was much younger than that of HCV mono-infected patients with the same complications.[9] This finding is compatible with reports from Western countries showing a faster progression of fibrosis[13] and earlier development of HCC.[14] A possibly interesting finding is that five patients (approximately 3% of patients whose serum HCV-RNA level was serially determined) cleared HCV-RNA from the serum without IFN treatment. Previous reports showed that some HIV-infected patients could spontaneously clear HCV-RNA.[15–17] The clearance of HCV among patients with chronic HCV infection is rare, although it has been

reported in Japan.[18] Three of the five patients had high HCV loads and low CD4+ T-lymphocyte counts, which are generally thought to be unfavorable for spontaneous HCV clearance. A difference in immune status of HIV-infected patients from HCV mono-infected patients may be involved in such an observation, although further studies are awaited.

In summary, our study demonstrated that approximately 20% of HIV-infected patients are co-infected with HCV. Some of the HIV–HCV co-infected patients had advanced liver disease such as ascites, encephalopathy or HCC at a younger age than HCV mono-infected patients, suggesting that the progression of liver disease may be more rapid in HIV–HCV co-infected patients than in HCV-mono-infected ones. Treatments with regimens including IFN, which may improve liver function and decrease liver-related death, should be considered in HIV–HCV co-infected patients.

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CLINICAL INVESTIGATION

Transarterial Infusion Chemotherapy Using Cisplatin-Lipiodol Suspension With or Without Embolization for Unresectable Hepatocellular Carcinoma

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Abstract We evaluate the long-term prognosis and prognostic factors in patients treated with transarterial infusion chemotherapy using cisplatin-lipiodol (CDDP/LPD) suspension with or without embolization for unresectable hepatocellular carcinoma (HCC). Study subjects were 107 patients with HCC treated with repeated transarterial infusion chemotherapy alone using CDDP/LPD (adjusted as CDDP 10mg/LPD 1ml). The median number of transarterial infusion procedures was two (range, one to nine), the mean dose of CDDP per transarterial infusion chemotherapy session was 30 mg (range, 5.0–67.5 mg), and the median total dose of transarterial infusion chemotherapy per patient was 60 mg (range, 10–390 mg). Survival rates were 86% at 1 year, 40% at 3 years, 20% at 5 years, and 16% at 7 years. For patients with >90% LPD accumulation after the first transarterial infusion chemotherapy, rates were 98% at 1 year, 60% at 3 years, and 22% at 5 years. Multivariate analysis identified >90% LPD accumulation after the first transarterial infusion chemotherapy ($p = 0.001$), absence of portal vein tumor thrombosis (PVTT; $p < 0.001$), and Child-Pugh class A ($p = 0.012$) as independent determinants of survival. Anaphylactic shock was observed in two patients, at the fifth transarterial infusion chemotherapy session in one and the ninth in the other. In conclusion, transarterial infusion chemotherapy with CDDP/LPD appears to be a useful treatment option for patients with unresectable HCC without PVTT and in Child-Pugh class A. LPD accumulation after the first transarterial infusion chemotherapy is an important prognostic factor. Careful consideration should be given to the possibility of anaphylactic shock upon repeat infusion with CDDP/LPD.

Keywords Hepatocellular carcinoma · Transcatheter arterial chemoembolization · Cisplatin-lipiodol suspension · Arterial infusion chemotherapy · Prognosis

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common malignant tumors worldwide [1–4]. Recent advances in imaging and treatment modalities have resulted in a number of improvements in the prognosis of patients with HCC. Patients with small HCCs, for example, are commonly treated by surgical resection and locoregional therapy such as percutaneous ethanol injection (PEI), microwave coagulation therapy, laser ablation, and radiofrequency (RF) ablation, and these treatments are often associated with a satisfactory long-term prognosis [5–9]. However, these locoregional therapies are not suitable in all patients, mainly due to the presence of a large tumor, multiple HCC tumors, or a serious underlying chronic liver disorder.

Since the development of transcatheter arterial embolization (TAE) for HCC [10–12], intra-arterial treatments have been widely used for patients with unresectable HCC.

Among these, transcatheter arterial chemoembolization (TACE) using anticancer drugs mixed with lipiodol (LPD; Lipiodol Ultrafluide; Laboratoire Guerbet, Aulnay-Sous-Bois, France), which remains selectively in tumor tissue for extended periods of time, has now become one of the most effective treatment modalities for patients with unresectable HCC [13–27]. Randomized controlled trials recently confirmed the survival benefits of TACE in such patients [28, 29].

Various anticancer drugs have been used as TACE agents in the treatment of HCC, including doxorubicin hydrochloride (ADM) [13–16], epirubicin hydrochloride [17], mitomycin C (MMC) [13, 16], zinostatin stimalamer (SMANCS) [27], and cisplatin (*cis*-diaminedichloroplatinum; CDDP) [30–33]. However, the most effective of these anticancer drugs and protocols against HCC has yet to be identified. In particular, little or no information is available on the effects of TACE-CDDP/LPD on prognosis or on the factor(s) predictive of a response.

We conducted a retrospective study to determine the long-term prognosis of patients who received transarterial infusion chemotherapy with CDDP/LPD for unresectable HCC and identified the factor(s) predictive of long-term prognosis.

Materials and Methods

Patients

From June 2000 to December 2007, 526 patients with naïve HCC were admitted to our hospital. Of these, 323 patients were treated with transarterial infusion chemotherapy, 68 with surgical resection, 5 with living-donor liver transplantation (LDLT), 54 with RF ablation, 13 with PEI, 4 with RF ablation and PEI, 32 with hepatic arterial infusion chemotherapy, 3 with systemic chemotherapy, and 24 with conservative therapy. Of the 323 patients treated with transarterial infusion chemotherapy, 91 were later treated with surgical resection, 41 with RF ablation, 35 with transarterial infusion chemotherapy combined with PEI, 7 with LDLT, 7 with radiotherapy, 32 with hepatic arterial infusion chemotherapy, and 3 with a combination of systemic chemotherapy, leaving 107 patients treated with transarterial infusion chemotherapy alone for enrollment in this retrospective cohort study. The study group consisted of 75 men and 32 women ranging in age from 42 to 92 years (median, 73 years). Tests were positive for hepatitis C virus in 82 patients (78.8%) and for hepatitis B virus in 7 patients (6.7%). Seventy-five patients were classified as having Child-Pugh class A (72.1%) disease and 29 as Child class B disease (27.9%). Median total bilirubin level was 1.0 mg/dl, and median serum albumin was 3.6 g/dl. Tumor staging was defined based on the tumor node metastasis staging system of the Liver Cancer Study Group of Japan (LCSGJ): stage I (fulfilling three intrahepatic conditions: solitary, <2 cm, no vessel invasion; $n = 9$ [9%]), stage II (two of the three intrahepatic conditions; $n = 41$ [38%]), stage III (one of the three intrahepatic conditions; $n = 53$ [50%]), stage IVa (none of the three intrahepatic conditions, with no distant metastases or any intrahepatic conditions with lymph node metastases), and stage IVb (any intrahepatic condition with distant metastases; stage IV, $n = 4$ [3%]) [34]. The median value of the maximum diameter of the main tumor was 30 mm (range, 6–130 mm). Forty-three (40%) patients had a solitary tumor, 35 (33%) had two or three tumors, and 29 (27%) had four or more tumors. The clinical characteristics of the study group are summarized in Table 1. The study was conducted in accordance with the Declaration of Helsinki. The study protocol was approved by the ethics committee of our hospital, and written informed consent was obtained from all participating patients.

Preparation of Chemotherapeutic Agents

LPD was mixed at 1 ml per 10 mg CDDP powder. Because CDDP powder was not available for clinical use in Japan from June 2000 to December 2004, we prepared CDDP powder from a commercially available CDDP solution (Randa; Nippon Kayaku, Tokyo) as described in our previous study [35]. After it became available, from December 2004 to December 2007, we mixed CDDP powder with

Table 1 Characteristics of 107 patients who underwent repeated transarterial infusion chemotherapy using a cisplatin/lipiodol suspension for unresectable hepatocellular carcinoma

Age, yr[a]	73 (42–92)
Gender, male/female	75/32
Etiology, HCV/HBV/others	82/7/18
Child-Pugh class, A/B/C	75/29/3
T-bilirubin, mg/dl[a]	1.0 (0.2–5.4)
Albumin, g/dl[a]	3.6 (2.4–4.7)
Tumor stage, T1/T2/T3/T4	9/41/53/4
Tumor size, mm[a]	30 (6–130)
Tumor number, 1/2 or 3/>3	43/35/29
Tumor portal vein thrombus, present/absent	3/104
α-Fetoprotein, ng/ml[a]	32.2 (5–35,610)
Des-γ-carboxy prothrombin, mAU/ml[a]	167 (10–11,600)
TAE, with/without	62/45
Period of follow-up, mo[a]	13 (1–92)

Note: *HCV* hepatitis C virus, *HBV* hepatitis B virus, *TAE* transcatheter arterial embolization

[a] Data are median (range)

LPD (IA-call; Nippon Kayaku). The particle size of CDDP powder is 28.5 μm.

Imaging and Confirmation of Diagnosis

Pretreatment imaging studies included abdominal ultrasonography (US), contrast-enhanced dynamic CT, dynamic magnetic resonance (MR) imaging, digital subtraction angiography (DSA), angiography combined with CT during arterial portography (CTAP), and hepatic arteriography (CTHA). All tumors were diagnosed by distinctive findings on US, dynamic CT and/or dynamic MR imaging, DSA, CTAP, and CTHA. Diagnosis was confirmed by early enhancement in the arterial phase and hypoattenuation in the portal venous or equilibrium phase on contrast-enhanced dynamic CT or dynamic MR images, or by hypoattenuation on CTAP and hyperattenuation on CTHA. In addition, changes in serum tumor markers (α-fetoprotein [AFP] or des-γ-carboxy prothrombin) were used to support the imaging-based diagnosis.

Transarterial Infusion Chemotherapy with or Without Embolization

Transarterial infusion chemotherapy was performed through the femoral artery under local anesthesia using the technique of Seldinger. An angiographic catheter was inserted into the hepatic feeding artery of the segment or subsegments containing the target tumor under CT scan during hepatic arteriography and arterial portography. We used a CDDP/LPD suspension as an anticancer drug. The tumor vessels were evaluated by CTHA scans during hepatic arteriography. Dosage was based on tumor size, and injection was discontinued based on the full accumulation of iodized oil in the tumor vessels and the degree of visualization of the portal vein during injection on fluoroscopy. The accumulation of iodized oil in the tumor was evaluated by CTHA scan; if accumulation in the tumor was poor, other vessels were tested, and when a vessel was identified as a feeding vessel, CDDP/LPD was added to the infusion. CDDP/LPD was not injected into the right hepatic artery, left hepatic artery, or proper hepatic artery.

A gelatin sponge was used for embolization (Gelpart; Nippon Kayaku, Tokyo), cut into 1- or 2-mm cubes, depending on the vascular diameter. The gelatin sponge was used after arterial infusion chemotherapy in patients who had a membrane-covered lesion and a segmental lesion in the periphery. Most patients were treated by arterial infusion chemotherapy in principle, but a gelatin sponge was not used in all patients, particularly those with chronic liver failure. A gelatin sponge was not used on the right hepatic artery, left hepatic artery, or proper hepatic artery. The angiographic endpoint of gelatin sponge embolization was very mild embolization. Extrahepatic collateral arteries which supplied tumors were also embolized.

The fluid replacement volume was 3000 ml/day on the day of treatment and 1000 ml/day for the next 2 days.

Criteria for Evaluation of the Therapeutic Effect of Transarterial Infusion Chemotherapy with or Without Embolization

The efficacy of transarterial infusion chemotherapy was evaluated by CT at 3 months after treatment, as follows: when LPD was seen in >90% of the tumor, efficacy was considered grade I; in 50% to 90% of the tumor, grade II; and in <50% of the tumor, grade III [35]. Grading for LPD retention was based on quantitative measurement of tumor diameter in all tumors, based on the assumption that the tumor portion with retained LPD was necrotic tissue. The percentage of LPD accumulation in the target tumor was graded by two radiologists blinded to clinical status. Discrepancies between the two observers were resolved by adopting the lowest grade of assessment.

Follow-Up Protocol

Concentrations of serum tumor markers, including AFP and des-γ-carboxy prothrombin, were measured once a month after transarterial infusion chemotherapy; follow-up US was performed every 3 months; and CT or MR imaging was performed every 6 months. Patients showing an increase in tumor markers, diminution of LPD accumulation, or new nodules remote from the treated nodules were readmitted for an additional round of transarterial infusion chemotherapy using the same procedure. On follow-up, patients treated with transarterial infusion chemotherapy who did not show complete uptake of LPD (i.e., those classified as grade I), but did show the presence of a viable tumor, namely, by arterial phase enhancement on CT/MR, were retreated with transarterial infusion chemotherapy within 3–6 months of the first treatment. Patients with tumor progression, appearance of PVTT, and liver failure were excluded from TACE.

Complications

Major complications were defined in accordance with the definitions established by the Society of Interventional Radiology as hemorrhage requiring transfusion, liver abscess requiring percutaneous drainage, bile duct injury requiring biliary drainage, pleural effusion requiring thoracocentesis, hepatic failure, and death [36]. In all patients, the following laboratory tests were conducted before treatment and 1, 3, and 7 days after and 1 month after

treatment: serum transaminases, bilirubin, alkaline phosphatase, albumin, creatinine, and complete blood cell count. Adverse reactions were assessed using the National Cancer Institute Common Toxicity Criteria (NCI-CTC; version 3.0) [37].

Statistical Analysis

Data were collected and calculated at the end of the study and statistically analyzed on April 1, 2008. Cumulative survival rate was calculated from the initial date of transarterial infusion chemotherapy therapy and assessed by the Kaplan–Meier life-table method, with differences evaluated by the log rank test. Univariate analysis of predictors of survival was assessed by the Kaplan–Meier life-table method, and differences were evaluated by the log rank test. Multivariate analysis of predictors of survival was assessed by the Cox proportional hazards model. Statistical significance was defined as a p value <0.05. We also calculated hazard ratios and 95% confidence intervals (95% CI). All p values <0.05 in two-tailed tests were considered significant. Variables that achieved statistical ($p < 0.05$) or marginal significance ($p < 0.10$) in univariate analysis were entered into a multiple Cox proportional hazards model to identify significant independent factors. Parameters used for the prediction of survival were LPD accumulation, tumor number, PVTT (present or absence), Child-Pugh class, AFP, age, gender, etiology, TAE (with or without embolization), and tumor size. All analyses were performed with SPSS software (version 16; SPSS, Chicago, IL).

Results

Therapeutic Effects of Transarterial Infusion Chemotherapy-CDDP/LPD

The median number of transarterial infusion chemotherapy procedures per patient was two (range, one to nine). The mean dose of CDDP per single session of transarterial infusion chemotherapy was 30 mg (range, 5.0–67.5 mg), and the median total dose of CDDP per patient was 60 mg (range, 10–390 mg). LPD accumulation was evaluated after the first transarterial infusion chemotherapy: grade I was recorded in 58 patients (55%), grade II in 36 (33%), and grade III in 13 (12%) (Table 2).

Survival Rates

Cumulative survival curves of patients treated with TACE using CDDP/LPD suspension for unresectable HCC. Survival rates were 86% at 1 year, 40% at 3 years, 20% at 5 years, and 16% at 7 years. No significant difference in overall survival was seen between patients with and those without embolization ($p = 0.20$) (Fig. 1). Survival rate in patients assessed as grade I was 98% at 1 year, 60% at 3 years, and 22% at 5 years. Respective rates, in contrast, were 68%, 52%, and 22% in those assessed as grade II and 48%, 20%, and 0% in those assessed as grade III (Fig. 2). The probability of survival correlated with the extent of LPD accumulation in grades I and III ($p < 0.05$). Representative examples of patients with grades I and II are shown in Fig. 3.

Table 2 Transarterial infusion chemotherapy with a cisplatin/lipiodol suspension

No. of procedures[a]	2 (1–9)
Mean dose of CDDP per single session, mg[a]	30 (5–67.5)
Total dose of CDDP per single case, mg[a]	60 (10–390)
LPD accumulation of transarterial infusion chemotherapy, grades I/II/III	55%/33%/12%

[a] Data are median (range)

We then investigated the relationship between survival after the initiation of transarterial infusion chemotherapy and various clinicopathological variables by univariate analysis. Results showed that survival correlated significantly with grade I ($p = 0.001$), and AFP < 200 ng/ml ($p = 0.013$) (Table 3). grade I, absence of PVTT, Child-Pugh class A, number of tumors = 1, and AFP < 200 ng/ml were then entered into the multiple Cox proportional hazard model, which identified grade I ($p = 0.001$), absence of PVTT ($p < 0.001$) and Child-Pugh class A

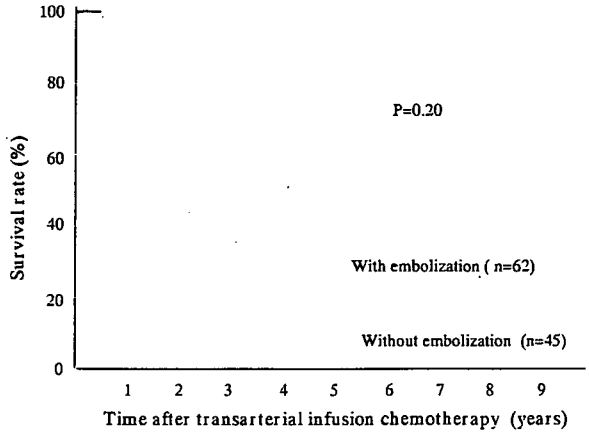

Fig. 1 Cumulative survival curves of patients treated with TACE using CDDP/LPD suspension for unresectable HCC. Survival rates were 96% at 1 year, 36% at 3 years, 24% at 5 years, and 24% at 7 years in with embolization groups and 72% at 1 year, 40% at 3 years, 16% at 5 years in without embolization groups. No significant difference in overall survival was seen between patients with and without embolization ($p = 0.20$)

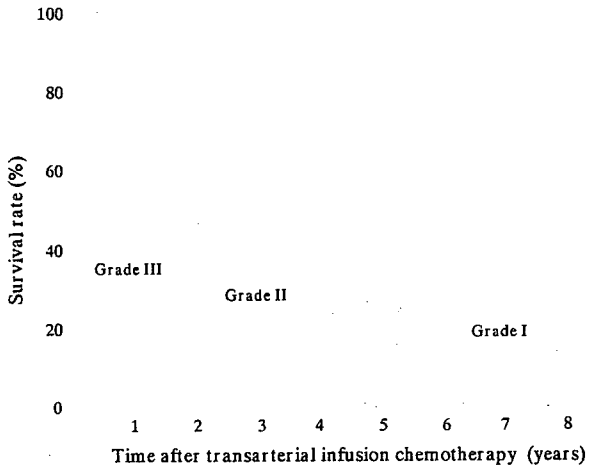

Fig. 2 Cumulative survival curves according to the degree of lipiodol (LPD) accumulation in the tumor. Survival rates of patients assessed as grade I were 98% at 1 year, 60% at 3 years, and 22% at 5 years. By comparison, rates in patients assessed as grade II were 68% at 1 year, 52% at 3 years, 22% at 5 years, and those in patients assessed as grade III were 48% at 1 year, 20% at 3 years, and 0% at 5 years. Survival probability correlated with degree of LPD accumulation between grade I and grade III ($p < 0.05$)

($p = 0.012$) as significant and independent determinants of survival.

Adverse Reactions and Complications

The total number of transarterial infusion chemotherapy procedures was 274. The most common adverse reactions were fever, nausea, and loss of appetite. Among patients with various NCI-CTC grade 2 adverse reactions, nausea and/or vomiting was the most common (96 patients; 35%), followed by grade 1 fever (71 patients; 26%), grade 3 thrombocytopenia (60; 22%), grade 2 abdominal pain (26; 9%), grade 2 liver dysfunction (26; 9%), grade 3 liver dysfunction (8; 3%), grade 3 renal dysfunction (2; 0.7%), grade 4 liver dysfunction (2; 0.7%), and anaphylactic shock (2; 0.7%). No intrahepatic biloma or liver abscess formation was seen. One patient received nine courses of transarterial infusion chemotherapy, with a total dose of CDDP of 310 mg. On injection of 15 mg/1.5 ml of CDDP/LPD suspension into the catheter on the ninth transarterial infusion chemotherapy, the patient experienced a decrease in systolic blood pressure from 110 to 78 mmHg and shortness of breath. He was successfully treated with oxygen and intravenous epinephrine and corticosteroid and was moved to the intensive care unit; he improved after 24 h and was transferred back to the general ward. Another patient received five courses of transarterial infusion chemotherapy, with a total dose of CDDP of 95 mg. Injection of 20 mg/2 ml of CDDP/LPD suspension into the catheter on the fifth transarterial infusion chemotherapy resulted in anaphylactic shock, but this patient also subsequently improved within 24 h.

Causes of Death

Forty-five of the 107 patients died during the study period. Causes of death were HCC-related (rupture of HCC) in 23 (51%), hepatic failure in 8 (18%), rupture of esophageal varices in 3 (7%), and other diseases in 11 (24%). No immediate or procedure-related death was seen within 30 days of infusion.

Discussion

The prognosis of patients with small HCC has improved markedly in recent years following the introduction of locoregional therapies. However, these therapies are not indicated in many patients due to large tumor size, multiple tumors, and poor underlying liver status. TACE has been widely used for these patients. Although various anticancer agents have been used as TACE agents for unresectable HCC, including ADM, epirubicin hydrochloride, MMC, SMANCS, and CDDP, the most effective anticancer drug for HCC remains to be defined. In vitro testing has indicated the efficacy of CDDP as suitable for TACE [38], but only a few reports have described the determinants of survival after initiation of TACE with CDDP/LPD suspension [39]. The purpose of the present study was to investigate the long-term prognosis of patients undergoing transarterial infusion chemotherapy with CDDP/LPD suspension for unresectable HCC and factors predictive of prognosis.

Overall survival rates in the 107 enrolled patients were 86% at 1 year, 40% at 3 years, 20% at 5 years, and 16% at 7 years. Ono et al. [39] reported survival rates of patients with unresectable HCC of 30% at 3 years with CDDP/LPD compared with 14% at 3 years with ADM. In other studies, survival rates at 3 years for unresectable HCC were 56% with ADM [40] and 32% with epirubicin hydrochloride [41]. Thus, the survival rate at 3 years achieved in the present study is very similar to those reported for ADM and epirubicin hydrochloride. The determinants of survival in the present study were grade I (>90% LPD accumulation in the first transarterial infusion chemotherapy), Child-Pugh grade A, and absence of PVTT; indeed, for patients with unresectable HCC free of PVTT who are rated as Child-Pugh grade A, a comparatively excellent long-term prognosis is expected for those who show >90% LPD accumulation after the first transarterial infusion chemotherapy.

CDDP is a potent anticancer drug against HCC in vitro. Using the 3-(4,5-dimethyithiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MMT) assay, Furukawa et al. [38]

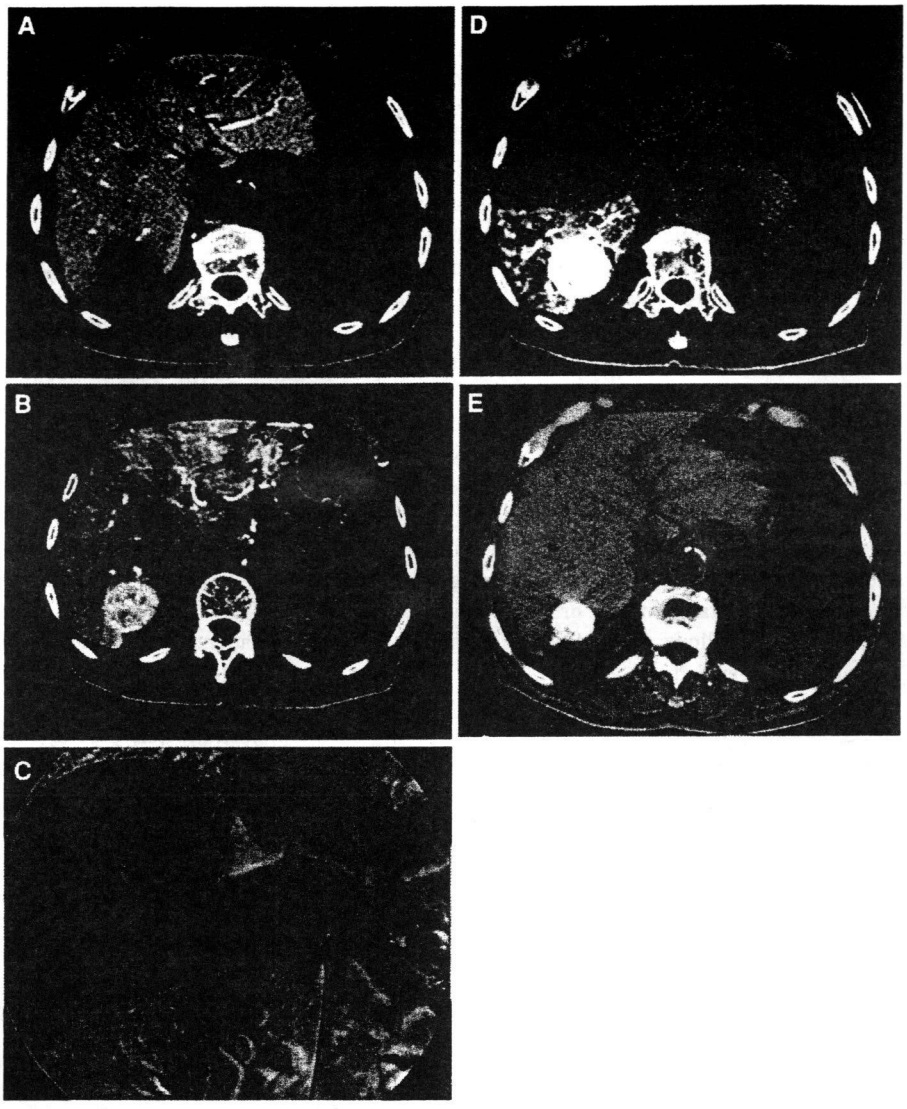

Fig. 3 Imaging studies in an 88-year-old man treated for unresectable HCC with TACE conducted between April 2006 and April 2008. Gelatin sponge embolization was conducted. **A** CTAP in April 2006. The HCC tumor (largest diameter, 4 cm) in S7 showed hypoperfusion on CTAP. **B** CTHA in April 2006 shows hyperenhancement of the same lesion. **C** DSA in April 2006 showing the same lesion. **D** CT taken 3 months after the first TACE. The lesion shows accumulation of LPD evaluated as grade I. **E** CT in April 2008 shows no recurrence 2 years later. Des-γ-carboxy prothrombin was decreased from 1100 to 10 mAU/ml. The patient remains alive and cancer-free at the time of writing

Table 3 Univariate and multivariate analyses of predictors of survival

	Univariate analysis (log-rank test): *p*-Value	Multivariate analysis (Cox proportional hazard model)		
		Hazard ratio	95% CI	*p*-Value
Grade I	0.001	0.335	0.172–0.654	0.001
Absence of PVTT	0.050	0.052	0.012–0.218	<0.001
Child-Pugh class A	0.083	0.436	0.228–0.834	0.012
No. of tumors = 1	0.095			
α-Fetoprotein <200 ng/ml	0.013			
Age <70	0.40			
Gender	0.80			
HBV/HCV/non-B non-C	0.33			
TAE (with/without)	0.20			
Tumor size <20 mm	0.42			

Note: *PVTT* portal vein tumor thrombosis, *TAE* transcatheter arterial embolization, *HBV* hepatitis B virus, *HCV* hepatitis C virus

reported the in vitro chemosensitivity of HCC to seven anticancer drugs as follows: ADM, 30%; CDDP, 20%; MMC, 17.5%; 5-fluorouracil, 12.5%; methotrexate, 5.4%; etoposide, 0%; and CPT-11, 0%. This indicates that ADM and CDDP are the most effective anticancer drugs for HCC in vitro. In their study, however, Kamada et al. [35] reported that the survival rate was significantly better for the CDDP/LPD group than for the ADM/LPD group. Comparison of the effects of and long-term prognosis for these anticancer drugs when used as TACE agents in randomized control trial studies is required.

LPD accumulation >90% after the first transarterial infusion chemotherapy was an independent determinant of survival. The proportion of patients who achieved this after the first transarterial infusion chemotherapy (55%) in the present study was higher than the 15% reported in our previous study [35]. This difference might be due to our present use of angiography combined with CT during arterial portography and hepatic arteriography, which provides better evaluation of drug accumulation in real time and, hence, allows the addition of an additional dose or drug when needed. In addition, CDDP/LPD was not injected into the right hepatic artery, left hepatic artery, or proper hepatic artery.

It seems that grading LPD uptake serves instead to represent a method to assess underlying tumor biology. Favorable tumor biology manifests with tumor necrosis and a high degree of LPD uptake, such as the case shown in Fig. 3, while unfavorable tumor biology results in lesser degrees of tumor necrosis and secondarily lower LPD uptake. It is doubtless that the effects of TACE are affected mainly by embolization with LPD and gelatin sponge. However, no significant difference in overall survival was seen between patients with and those without embolization in our study.

Ikeda et al. also reported that although transcatheter arterial infusion chemotherapy had a stronger antitumor effect than transarterial infusion chemotherapy, it did not significantly improve survival [42]. In contrast, Yamamoto et al. reported that complete embolization after injection of cisplatin-lipiodol suspension resulted in higher survival than incomplete embolization [32]. We consider that gelatin sponge embolization was locally effective in the tumor, but because survival rates were also related to liver function, gelatin sponge embolization was not a significant prognostic factor in this study.

Although we used a CDDP/LPD suspension in the present study, Takaki et al. recently reported that LPD retention was better with the emulsion than with the suspension [43]. Evaluation of the best mixing method for CDDP and LPD requires long-term investigation.

Analysis of adverse reactions and complications with transarterial infusion chemotherapy-CDDP/LPD showed minimal renal or liver dysfunction. This favorable finding may be due to selective infusion of the drug under CTAP and CTHA: because the injected area can be viewed directly under CTHA, the amount of injected drug that can cause damage to noncancer tissue is minimal [44], and the mean dose of CDDP per single session of transarterial infusion chemotherapy was a relatively low 30 mg. Nevertheless, anaphylactic shock was observed in two (0.7%) patients. A recent review reported five patients with gynecological malignancies who experienced anaphylaxis to CDDP after receiving previously uncomplicated courses of this agent, with the hypersensitivity reaction following a median of seven courses [45, 46]. In our study, two patients experienced hypersensitivity, at the fifth and ninth courses, respectively, suggesting the need for caution when administering platinum agents to patients previously treated with the agent. Monitoring during CDDP/LPD injection is therefore warranted, and injection should be stopped at the first sign of symptoms.

In conclusion, transarterial infusion chemotherapy with CDDP/LPD appears to be a useful treatment option for patients with unresectable HCC without PVTT and in Child-Pugh class A. LPD accumulation after the first transarterial infusion chemotherapy is an important prognostic factor. Careful consideration should be given to the possibility of anaphylactic shock upon repeat infusion with CDDP/LPD.

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