state in the early years contributed to suppress the process of carcinogenesis, and that reactivation of hepatitis induced the progression of hepatic oncogenesis in the later years.

Among patients with a high platelet count and mild liver disease, IFN did not decrease the rate of hepatocarcinogenesis. IFN significantly decreased the carcinogenesis rate in patients with a low or intermediate platelet count. In view of the less effective rate and high adverse reaction rate by IFN in elderly patients, IFN therapy should be considered primarily for those with a low platelet count of 150,000/mm^3 or less. Because low platelet count was closely associated with advanced disease and high risk for carcinogenesis, treatment efficacy appeared prominent in the subgroup with low and intermediate platelet counts. The best candidates for IFN therapy were those with a low platelet count, also in regard to cost-effectiveness. Because a low platelet count is closely associated with advanced stages of liver disease, IFN therapy should be avoided for elderly patients with decompensated cirrhosis or severely decreased platelet count of less than 50,000/mm^3. A sustained virologic response improves clinical symptoms in decompensated cirrhosis,[31] but IFN often induces severe complications even in young patients with decompensated cirrhosis.[32] An elderly patient with hepatitis without decompensation can be a candidate for IFN therapy if careful, close hematologic monitoring is performed. Low-dose, intermittent, long-term IFN therapy also should be considered for these patients to obtain a sustained biochemical response without creating profound and irreversible side effects. Because elderly patients generally showed some difficulties with IFN treatment, our current study demonstrated practical information about carcinogenesis and the life expectancy of elderly patients with HCV and the order of priority in management of IFN for these patients. IFN administration is preferably considered and initiated at the age of 60 years or less to reduce side effects.

CONCLUSIONS

IFN for a subgroup with low and intermediate platelet counts had significant advantages in regard to hepatocarcinogenesis and survival of elderly patients with chronic HCV.

References

1. Bruix J, Calvet X, Costa J, et al. Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet.* 1989;2:1004-1006.
2. Colombo M, Kuo G, Choo QL, et al. Prevalence of antibodies to hepatitis C virus in Italian patients with hepatocellular carcinoma. *Lancet.* 1989;2:1006-1008.
3. Ikeda K, Saitoh S, Koida I, et al. A multivariate analysis of risk factors for hepatocellular carcinogenesis: a prospective observation of 795 cases with viral and alcoholic cirrhosis. *Hepatology.* 1993;18:47-53.
4. Williams I. Epidemiology of hepatitis C in the United States. *Am J Med.* 1999;107:2S-9S.
5. Alter MJ. Epidemiology of hepatitis C virus infection. *World J Gastroenterol.* 2007;13:2436-2441.
6. Davis GL, Balart LA, Schiff ER, et al. Treatment of chronic hepatitis C with recombinant interferon alfa. A multicenter, randomized, controlled trial. *N Engl J Med.* 1989;321:1501-1506.
7. Di Bisceglie AM, Martin P, Kassianides C, et al. Recombinant interferon alfa therapy for chronic hepatitis C: a randomized, double-blind, placebo-controlled trial. *N Engl J Med.* 1989;321:1506-1510.
8. Nishiguchi S, Kuroki T, Nakatani S, et al. Randomized trial of effects of interferon-alpha on incidence of hepatocellular carcinoma in chronic active hepatitis C with cirrhosis. *Lancet.* 1995; 346:1051-1055.
9. Mazzella G, Accogli E, Sottili S, et al. Alpha interferon treatment may prevent hepatocellular carcinoma in HCV-related liver cirrhosis. *J Hepatol.* 1996;24:141-147.
10. Kasahara A, Hayashi N, Mochizuki K, et al. Risk factors for hepatocellular carcinoma and its incidence after interferon treatment in patients with chronic hepatitis C. Osaka Liver Disease Study Group. *Hepatology.* 1998;27:1394-1402.
11. Niederau C, Lange S, Heintges T, et al. Prognosis of chronic hepatitis C: results of a large, prospective cohort study. *Hepatology.* 1998;28: 1687-1695.
12. International Interferon-alpha Hepatocellular Carcinoma Study Group. Effect of interferon-alpha on progression of cirrhosis to hepatocellular carcinoma: a retrospective cohort study. *Lancet.* 1998;351:1535-1539.
13. Ikeda K, Saitoh S, Arase Y, et al. Effect of interferon therapy on hepatocellular carcinogenesis in patients with chronic hepatitis type C: a long-term observation study of 1643 patients using statistical bias correction with proportional hazard analysis. *Hepatology.* 1999;29: 1124-1130.
14. Tsubota A, Chayama K, Ikeda K, et al. Factors predictive of response to interferon-alpha therapy in hepatitis C virus infection. *Hepatology.* 1994;19:1088-1094.
15. Hagiwara H, Hayashi N, Mita E, et al. Quantitative analysis of hepatitis C virus RNA in serum during interferon alpha therapy. *Gastroenterology.* 1993;104:877-883.
16. Floreani A, Monola E, Carderi I, et al. Are elderly patients poor candidates for pegylated interferon plus ribavirin in the treatment of chronic hepatitis C? *J Am Geriatr Soc.* 2006;54:549-550.
17. Davis GL, Albright JE, Cook SF, Rosenberg DM. Projecting future complications of chronic hepatitis C in the United States. *Liver Transpl.* 2003;9:331-338.
18. Taura N, Hamasaki K, Nakao K, et al. Aging of patients with hepatitis C virus-associated hepatocellular carcinoma: long-term trends in Japan. *Oncol Rep.* 2006;16:837-843.
19. Yoshizawa H, Tanaka J, Miyakawa Y. National prevention of hepatocellular carcinoma in Japan based on epidemiology of hepatitis C virus infection in the general population. *Intervirology.* 2006;49:7-17.
20. Alessi N, Freni MA, Spadara A, et al. Efficacy of interferon treatment (IFN) in elderly patients with chronic hepatitis C. *Infez Med.* 2003;11: 208-212.
21. Koyama R, Arase Y, Ikeda K, et al. Efficacy of interferon therapy in elderly patients with chronic hepatitis C. *Intervirology.* 2006;49:121-126.
22. Nudo CG, Wong P, Hilzenrat N, Deschenes M. Elderly patients are at greater risk of cytopenia during antiviral therapy for hepatitis C. *Can J Gastroenterol.* 2006;20:589-592.
23. Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J Am Stat Assoc.* 1958;53:457-481.
24. Cox DR. Regression models and life tables. *J R Stat Soc.* 1972;34: 248-275.
25. SPSS Inc. *SPSS for Windows Version 11.0 Manual.* Chicago, IL: SPSS Inc.; 2001.
26. Bresci G, Del Corso L, Romanelli AM, et al. The use of recombinant interferon alfa-2b in elderly patients with anti-HCV-positive chronic active hepatitis. *J Am Geriatr Soc.* 1993;41:857-862.

27. Horiike N, Masumoto T, Nakanishi K, et al. Interferon therapy for patients more than 60 years of age with chronic hepatitis C. *J Gastroenterol Hepatol.* 1995;10:246-249.
28. Thabut D, Le Calvez S, Thibault V, et al. Hepatitis C in 6,865 patients 65 yr or older: a severe and neglected curable disease? *Am J Gastroenterol.* 2006;101:1260-1267.
29. Honda T, Katano Y, Urano F, et al. Efficacy of ribavirin plus interferon-alpha in patients aged > or = 60 years with chronic hepatitis C. *J Gastroenterol Hepatol.* 2007;22:989-995.
30. Arase Y, Ikeda K, Suzuki F, et al. Long-term outcome after interferon therapy in elderly patients with chronic hepatitis C. *Intervirology.* 2007;50:16-23.
31. Iacobellis A, Siciliano M, Perri F, et al. Peginterferon alfa-2b and ribavirin in patients with hepatitis C virus and decompensated cirrhosis: a controlled study. *J Hepatol.* 2007;46:206-212.
32. Nevens F, Goubau P, Van Eyken P, et al. Treatment of decompensated viral hepatitis B-induced cirrhosis with low doses of interferon alpha. *Liver.* 1993;13:15-19.

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Occult hepatitis B virus infection increases hepatocellular carcinogenesis by eight times in patients with non-B, non-C liver cirrhosis: a cohort study

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SUMMARY. An impact of serum hepatitis B virus (HBV) DNA on hepatocarcinogenesis has not been investigated in a cohort of patients with non-B, non-C cirrhosis. Eighty-two consecutive Japanese patients with cirrhosis, who showed negative hepatitis B surface antigen and negative anti-hepatitis C virus, were observed for a median of 5.8 years. Hepatitis B virus core (HBc) region and HBx region were assayed with nested polymerase chain reaction. Both of HBc and HBx DNA were positive in 9 patients (11.0%) and both were negative in 73. Carcinogenesis rates in the whole patients were 13.5% at the end of the 5th year and 24.6% at the 10th year. The carcinogenesis rates in the patients with positive DNA group and negative DNA group were 27.0% and 11.8% at the end of the 5th year, and 100% and 17.6% at the 10th year, respectively ($P = 0.0078$). Multivariate analysis showed that men ($P = 0.04$), presence of HBc and HBx DNA (hazard ratio: 8.25, $P = 0.003$), less total alcohol intake ($P = 0.010$), older age ($P = 0.010$), and association of diabetes ($P = 0.005$) were independently associated with hepatocellular carcinogenesis. Existence of serum HBV DNA predicted a high hepatocellular carcinogenesis rate in a cohort of patients with non-B, non-C cirrhosis.

Keywords: hepatitis B virus, hepatocellular carcinogenesis, liver cirrhosis, occult hepatitis B virus infection, proportional hazard model.

INTRODUCTION

Hepatocellular carcinoma (HCC) is a leading cause of death in many parts of sub-Saharan Africa and Asia [1,2]. It is also one of the most common neoplasms in Japan [3]. Hepatitis B virus (HBV) infection is the primary cause of cirrhosis and HCC and one of the major causes of death globally [4]. Needless to say, a cohort of patients with HBV-related chronic hepatitis and cirrhosis has a significantly high risk for HCC development [5–7]. In our retrospective cohort studies concerning HBV-related disease, cumulative hepatocellular carcinogenesis rates in chronic hepatitis ($n = 610$) and cirrhosis ($n = 180$) were 2.1% and 7.2% at the end of the 5th year, and 4.9% and 27.2% at the 10th year, respectively [5,7]. Abundant epidemiological and molecular biological evidence shows that HBV is an important factor in the development of HCC [8–10], but the precise role of HBV in the oncogenesis is still unknown.

HBV infection is usually diagnosed when the circulating hepatitis B surface antigen (HBsAg) is detected. However, the availability of highly sensitive molecular biology techniques has allowed the identification of HBV infection in HBsAg-negative individuals with or without circulating antibodies to HBsAg and/or hepatitis B core antigen (anti-HBc) [11–16]. Much evidence suggests that this so-called occult HBV infection is highly prevalent in a number of patient subgroups including those with HCV infection [16,17], cryptogenic advanced liver fibrosis [18] and HCC [17,19–27]. Although Marusawa *et al.* [28] and Uetake *et al.* [29] described the relationship between anti-HBc and HCC appearance rate in each study, impact of occult HBV infection on carcinogenesis cannot be evaluated because of lack of HBV DNA assay. As all the previous studies were performed as a pilot study or a case-controlled one, actual risk ratio of occult HBV infection for hepatocellular carcinogenesis has not been reported in a cohort study until now.

Abbreviations: AFP, alpha-fetoprotein; ALT, alanine transaminase; AST, aspartic transaminase; HBsAg, hepatitis B surface antigen; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; PCR, polymerase chain reaction.

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We, therefore, analysed a retrospective cohort of consecutive patients with cirrhosis for a long period, in order to elucidate the influence of occult HBV infection on the carcinogenesis rate from non-B, non-C cirrhosis.

PATIENTS AND METHODS

Patients

Among 103 consecutive patients diagnosed as having non-B, non-C cirrhosis by peritoneoscopic liver biopsy at Toranomon Hospital, Tokyo, Japan in the period from 1976 to 1998, initial frozen sera at the time of the diagnosis of cirrhosis were available for the assay of HBV DNA in 82 patients (79.6%). The cohort of 82 patients was retrospectively observed for a long period. All the patients showed negative HBsAg, negative anti-hepatitis C virus (HCV) and negative HCV RNA. Patients with a possible association of HCC at the time of the diagnosis of cirrhosis were strictly excluded from this study. No patient received interferon or other antiviral therapy after the diagnosis of cirrhosis.

Background and laboratory data of the patients

There were 67 men and 15 women aged 34–80 with a median age of 58 years. A total of 47 patients (57.3%) had a history of alcohol intake of more than 500 kg until the diagnosis of liver cirrhosis. Fifteen patients (18.3%) had decompensated cirrhosis with ascites, a history of encephalopathy, or both. The median value of indocyanine green retention rate at 15 min (ICG R15) was 33% (range, 7–75%), and total bilirubin concentration was 1.3 mg/dL (range 0.4–20.9 mg/dL).

Measurement of hepatitis virus markers

Hepatitis virus markers were assayed using frozen sera at −80 °C. All sera were tested for HBsAg (radioimmunoassay, Dainabot, Tokyo, Japan), anti-HCV (second-generation anti-HCV, enzyme-linked immunosorbent assay, Dainabot), and HCV RNA with reverse transcription-nested polymerase chain reaction (PCR).

HBV DNA was analysed for the region of HBc and HBx by sensitive nested PCR according to Yotsuyanagi *et al.* [30]. Fifty microlitres of STE solution [100 mmol/L Tris–HCl (pH 8.0), 100 mmol/L NaCl, 2 mmol/L ethylenediaminetetraacetic acid (pH 8.0), and 0.2% sodium dodecyl sulphate] with 20 μg of proteinase K (Boehringer, Mannheim, Germany) were added to serum samples. Mixed samples were then incubated for 2 h at 55 °C. DNA was extracted twice with phenol/chloroform, once with chloroform, and precipitated with ethanol. The DNA pellet was dissolved in 25 μL of TE buffer [10 mmol/L Tris–HCl (pH 8.0) and 1 mmol/L ethylenediaminetetraacetic acid (pH 8.0)].

Prepared DNA was subjected to amplification using nested PCR technique. HBV DNA was amplified using two independent pairs of primers, with each primer complementary to sequences in the X or core region of the HBV genome [30]. Amplification was performed using a thermal cycler for a total of 40 cycles, with each cycle consisting of 94 °C for 1 min, 55 °C for 1 min and 72 °C for 1 min, in 100 μL of reaction mixture containing 200 mmol/L of each dNTP, 1X PCR buffer [50 mmol/L KCl, 10 mmol/L Tris–HCl (pH 8.3), 1.5 mmol/L $MgCl_2$ and 0.001% (w/v) gelatine], and 2 units of Ampli-Taq polymerase (Perkin Elmer Cetus Corp., Norwalk, CT, USA). The PCR products were separated in a 2% agarose gel and transferred to a nylon membrane (Schleicher and Schuell, Dassel, Germany). The membrane was then probed with digoxigenin-labelled oligonucleotides, which hybridize specifically with the core or X gene. Results were considered valid only if the same results were obtained in at least two separate experiments.

We considered the cases with positivity in at least two different viral genomic regions as HBV DNA positive. Appropriate negative controls were included in each PCR. The limit of sensitivity of our nested PCR methods ranged from 10 to 1 genome equivalents/mL.

Follow-up of patients

Follow-up of the patients was made on a monthly or bimonthly basis after diagnosis of cirrhosis by monitoring alpha-fetoprotein (AFP) and other biochemical data. Imaging diagnosis was made at least once a year for each patient with CT or US. After 1988, in order to detect HCC earlier, imagings were done three or more times per year in a majority of patients.

No patient underwent interferon therapy after the diagnosis of cirrhosis, but some of the patients received an oral or intravenous administration of medicinal herbs during the follow-up period.

All patients were finally evaluated in November 2004. The cases lost to follow-up were 13 (15.9%). The median observation period of the total patients was 5.8 years with a range of 0.1–34.8 years.

Statistical analysis

Differences of background features and laboratory data between the patients with and without HBV DNA were analysed by chi-square test, Fisher's exact test and Mann–Whitney's *U*-test. The time between diagnosis of cirrhosis and appearance of HCC was analysed using the Kaplan–Meier technique [31] and differences in curves were tested using log-rank test [32]. Those patients who had been lost to follow-up were regarded as censored data at the time of missing in the statistics. Independent risk factors associated with the appearance rate of HCC were studied using the stepwise Cox regression analysis [33]. Potential risk factors

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assessed for hepatocellular carcinogenesis included the following 18 variables: age, sex, association of diabetes mellitus, total alcohol intake, history of cigarette smoking, family history of liver disease, history of blood transfusion, state of cirrhosis (presence of ascites and/or a history of encephalopathy), HBc DNA, HBx DNA, aspartic transaminase (AST), alanine transaminase (ALT), albumin, bilirubin, globulin, AFP, platelet, and ICG R15. A probability less than 0.05 was considered as significant. Data analysis was performed using computer program SPSS version 11 [34].

RESULTS

HCC appearance rate in all the patients

During the observation period, HCC appeared in 16 patients (19.5%). Median interval between the diagnosis of cirrhosis and HCC was 5.6 years (range 0.7–15.6 years) in the patients with HCC development. The cumulative HCC appearance rate in the 82 patients was 13.5% at the end of the fifth year after the diagnosis of cirrhosis, 24.6% at the end of tenth year, 33.3% at the 15th year, and 41.6% at the end of 20th year.

HCC appearance rates according to serum HBV DNA

Among the 82 patients, 9 patients (11.0%) showed positive serum HBV DNA and 73 (89.0%) negative HBV DNA. The former 9 patients had both HBc DNA and HBx DNA, and the latter 73 had neither of them. Table 1 summarizes the profiles and laboratory data of each group. There was no demographic difference between the two groups. There was also no statistically significant difference between them except for ALT value, which was lower in the patient group with positive HBV DNA ($P = 0.028$).

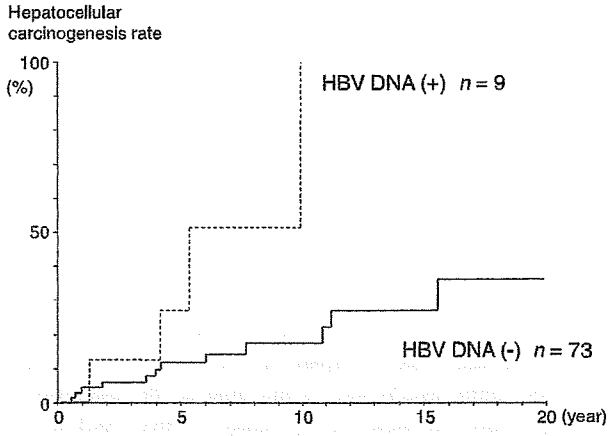

Fig. 1 Hepatocellular carcinogenesis curves of the patients with and without serum hepatitis B virus DNA. Carcinogenesis rates were 12.5% and 6.0% at the end of the third year, 27.0% and 11.8% at the fifth year, and 100% and 17.6% at the tenth year, respectively.

Figure 1 shows the curves of crude HCC appearance rate in the two patients group with and without serum HBV DNA. The third-year HCC appearance rates in the patients with and without DNA were 12.5% and 6.0%, the 5th-yr rates 27.0%, 11.8%, the tenth-yr rates 100% and 17.6%, respectively. The HCC appearance rate of the patient group

Table 1 Demography and laboratory data of patients with and without serum hepatitis B virus DNA

	HBV DNA*		
	Positive (n = 9)	Negative (n = 73)	P
Demographic and background features			
Sex – men/women	8/1	59/14	0.55
Age (median, range)	51 (45–68)	58 (34–80)	0.44
History of transfusion	1 (11.1%)	14 (19.4%)	0.55
Alcohol intake of 500 kg or more	5 (55.6%)	42 (58.3%)	0.87
Diabetes mellitus	3 (33.3%)	15 (20.8%)	0.40
Observation period (years)	5.7 (1.0–21.0)	6.1 (0.1–34.8)	0.92
Laboratory data (median, range)			
ICG R15 (%)	34 (12–51)	32.5 (7–75)	0.78
AST (IU/L)	32 (17–86)	40.5 (14–184)	0.26
ALT (IU/L)	16 (9–43)	28.5 (4–160)	0.028
Albumin (g/dL)	3.8 (2.6–4.5)	3.6 (1.7–5.2)	0.20
Bilirubin (mg/dL)	0.9 (0.5–2.8)	1.3 (0.4–20.9)	0.14
Platelet ($\times 1000/mm^3$)	142 (67–232)	104 (27–647)	0.18
AFP (ng/mL)	5 (3–9)	6 (1–98)	0.38

ICG R15, indocyanine green retention rate at 15 min; AST, aspartic transaminase; ALT, alanine transaminase; AFP, alpha-fetoprotein. *HBV DNA was assessed for HBc and HBx DNA using polymerase chain reaction

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of positive HBV DNA was slightly higher than that of negative DNA (P = 0.0078, log-rank test).

Significance of serum HBV DNA in hepatocellular carcinogenesis

Cox proportional hazard model was performed for analysis of risk factors for liver carcinogenesis, using the 18 variables as mentioned above.

In the last step of stepwise regression analysis, the following five variables entered the model and could not be removed: sex (P = 0.005), serum HBV DNA (P = 0.003), past history of alcohol intake (P = 0.003), age (P = 0.035), and association of diabetes mellitus (P = 0.022) (Table 2). Accordingly, these five factors were significantly associated with hepatocellular carcinogenesis in the patients with non-B, non-C cirrhosis. Among them, gender was the strongest predictor of future HCC occurrence rate, indicating that male patients had 15.4 times as high carcinogenesis hazard as women patients. Similarly, positive HBV DNA (hazard ratio, 8.25) and little alcohol consumption of less than 500 kg (hazard ratio, 7.19) were the second and third strongest predictors for carcinogenesis, respectively. When the background factors of the cases were adjusted with the other significant factors, positive test for HBV DNA was significantly associated with the hepatocellular carcinogenesis rate.

Curves of carcinogenesis rates were generated from the multivariate analysis in an imaginary positive DNA group and an imaginary negative DNA group, with average sex ratio, average alcohol intake, average age and average association rate of diabetes (Fig. 2). The difference of the carcinogenesis curves indicated 'pure' impact of positive serum HBV DNA upon the carcinogenesis, which was adjusted with significant covariates assuming a standardized study group.

Table 2 Independent factors associated with liver carcinogenesis in the patients with non-B, non-C cirrhosis

Factors	Category	Hazard ratio (95% confidence interval)	P
Sex	Women	1	
	Men	15.4 (2.24–111.1)	0.005
Serum HBV DNA*	Negative	1	
	Positive	8.25 (2.01–33.93)	0.003
Total alcohol intake	≥500 kg	1	
	<500 kg	7.19 (1.98–26.32)	0.003
Age	<60 years	1	
	≥60 years	3.98 (1.10–14.42)	0.035
Diabetes mellitus	No	1	
	Yes	3.89 (1.22–12.47)	0.022

*Positive HBV DNA: positive for both HBc DNA and HBx DNA.

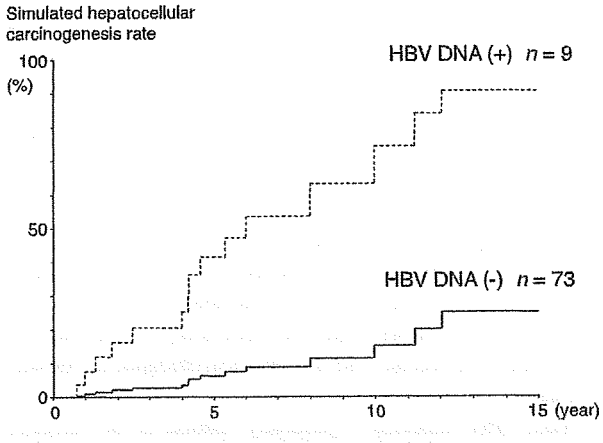

Fig. 2 'Adjusted' hepatocellular carcinogenesis rates in the positive HBV DNA group and the negative DNA group. Cox proportional hazard analysis showed that the carcinogenesis rate in the positive DNA group was significantly higher than that of the negative DNA group, when the other significant covariates were substituted with the same average parameters in the two groups.

Mortality and causes of death

During the observation period, 36 (43.9%) of 82 patients died: 5 (55.6%) of 9 patients in the positive DNA group and 31 (42.5%) of 73 patients in the negative DNA group. Cumulative survival rates in patients with and without HBV DNA were 78.8% and 74.1% at the end of the fifth year, 54.4% and 44.4% at the tenth year, 38.4% and 29.6% at the 15th year, and 33.6% and 29.6% at the 20th year, respectively. Although the survival rate in the positive HBV DNA group was lower than in the negative group, statistical significance was not shown.

Causes of death included liver failure due to liver cirrhosis in 21 (4 in positive DNA group and 17 in negative DNA group), progression of HCC in 7 patients (all in negative DNA group), and other causes in 8 (one in positive DNA group and 7 in negative DNA group).

DISCUSSION

Epidemiological and molecular virological studies in the 1970s and early 1980s established a strong aetiological association between chronic HBV infection and the hepatocellular carcinogenesis [35]. We also estimated annual carcinogenesis rates as 0.5% in chronic hepatitis and 3% in cirrhosis, from cohorts of biopsy-proven HBV disease [5,7].

Integration of HBV DNA has been reported in the majority of HBsAg positive HCCs since 1980s, and the fact suggested HBV might be oncogenic. Up to now, there is no evidence

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that HBV DNA is directly oncogenic and the mechanism by which chronic HBV infection leads to carcinogenesis remains unclear. Integration of HBV DNA may stimulate cellular pro-oncogenes or suppress growth-regulating genes [36]. Integration of HBV DNA, however, has been found in varied regions of the host chromosomes and no preferential and specific site has been identified until now. The other authors suggested that integration of HBV DNA could also induce carcinogenesis via transactivation of other oncogenes [37]. Both HBx protein and the truncated pre-S/S protein are potent transactivators and are commonly found in HCC tissue but their precise role in hepatocarcinogenesis remains unknown.

Occult HBV infection is generally defined as the detection of HBV DNA in the serum or liver tissue of patients who test negative for hepatitis B surface antigen [38–41]. Occult HBV infection was first reported in the early 1980s when hybridization techniques for the detection of HBV DNA became available. These studies showed that HBV DNA could be detected in HBsAg negative patients with HCC [42]. Recent studies using more sensitive techniques confirmed the close correlation between chronic occult HBV infection and carcinogenesis. Many authors demonstrated the relationship between occult HBV infection and hepatocellular carcinogenesis, mainly by a pilot study or a case–control study [17,19–27]. Shiota *et al.* [24] reported in their case studies without control group that serum of 18 out of 26 HCC patients without HBsAg and anti-HCV were positive for either S, C, or X region on PCR and southern blotting. Pollicino *et al.* [26] described that viral DNA was detected in 68 of 107 cases of HCC tissue (63.5%) and in 63 of 192 cases of chronic hepatitis tissue (32.8%), and concluded that occult HBV is a risk factor for development of HCC. The other authors also emphasized the high incidence of HBV DNA in either serum or HCC tissue compared with that of cases without HCC development. All the literatures, except one [43] from Taipei where HBV infection was endemic and prevalent, concluded that occult HBV infection was closely associated HCC development. However, precise risk or hazard ratio for carcinogenesis has not been reported.

Current study on this topic provided strong evidence of an association between occult HBV infection and HCC. In the patient cohort of non-B, non-C cirrhosis, occult HBV infection increased the future carcinogenesis rate with a hazard ratio of 8.25 (95% confidence interval, 2.01–33.93). It has been proposed that diagnosis of occult HBV infection be made only when HBV DNA can be detected using at least two sets of primers from different areas of the HBV genome in duplicate assay [38,39]. Appropriate negative controls must be included in each assay and specificity of the amplification reaction confirmed by sequencing of the amplicons. Using this strict criterion, occult HBV infection was found in 9 (11.0%) of 82 Japanese patients with non-B, non-C cirrhosis. Background features of the nine patients with serum HBV DNA showed a slightly younger age, a lower ALT, a slightly lower bilirubin, and a slightly higher platelet count (Table 1). Although all these demographic and laboratory findings were considered to favour low carcinogenetic risk, the patients with cryptic HBV DNA infection developed HCC more frequently. After adjustment of these background covariates in the multivariate analysis, positivity of serum HBV DNA proved to be an independent risk factor for hepatocarcinogenesis (Table 2).

As this retrospective cohort consisted of only cirrhosis as an advanced liver disease, and as it included both alcoholic and non-alcoholic cirrhosis, the hazard ratio of 8.25 could not be applied for varied stages and varied aetiologies of liver disease. In order to elucidate the impact of occult HBV infection on carcinogenesis, future studies should be performed also in the other cohort of chronic liver disease, such as HCV-related disease. Although anti-HBc and anti-HBs antibody were measured in a small numbers of the patients, an exact relationship between serum HBV DNA and serum positivity of anti-HBc antibody was not analysed in this study. When we tested anti-HBc antibody in a small part of subjects, 3 of 6 patients (50.0%) with positive HBV DNA had serum anti-HBc antibody and 7 of 19 patients (36.8%) without HBV DNA had anti-HBc (Fisher's exact test, $P = 0.69$). For the convenience of clinical circumstance and practical usefulness, significance of positive anti-HBc on carcinogenesis risk should be elucidated through a large-scale cohort study with an identical assay for anti-HBc antibody.

Although a lot of epidemiological and clinicopathological evidence of the relationship has been published, precise role of occult HBV in this setting has been still unclear. Patients with occult hepatitis B overlap with those who previously have been classified as having recovered [44]. In fact, the distinction between recovery and occult hepatitis B is likely to be somewhat arbitrary, as recovery does not necessarily imply eradication of infection in all cases [30], but includes the possibility of complete suppression in some cases by a broad and vigorous immune response [44]. One of the most important clinical questions is whether occult hepatitis B merely represents a marker of past infection, or whether HBV genome persistence contributes to liver disease. It is very likely that occult HBV is a cofactor in the development of HCC. Several studies found that patients co-infected with HBV and HCV have increased risks of HCC compared with those with mono-infection. Our cohort studies [45] also showed that a risk factor of a history of heavy drinking interacted with HBV or HCV subtypes in a characteristic manner from the viewpoint of carcinogenesis in cirrhosis. The other important problem is whether occult HBV infection alone causes HCC. To address this question, studies on occult HBV infection in patients with HCC might provide details on other causes of chronic liver disease including nonalcoholic fatty liver disease, which may masquerade as cryptogenic cirrhosis, hemochromatosis, alfa-1-antitrypsin deficiency, and autoimmune liver disease [46]. Recently,

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Castillo *et al.* [47] reported a clinical state of occult HCV infection, which shows negative serum anti-HCV, negative serum HCV RNA, and positive HCV RNA in liver biopsy specimen. Although we did not tested the possibility of occult HCV infection in this study, future studies should be also aimed at the influence of latent HCV infection on hepatocarcinogenesis.

In conclusion, occult HBV infection significantly increased the incidence of hepatocellular carcinogenesis in patients with non-B, non-C cirrhosis. Although non-B, non-C cirrhosis seemed to include varied aetiology of liver disease, cryptic HBV infection should be taken account in the prediction of future HCC development.

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REFERENCES

1 Parkin DM, Stjernsward J, Muir CS. Estimates of worldwide frequency of twelve major cancers. *Bull World Health Organ* 1984; 62: 163–182.
2 Okuda K. Early recognition of hepatocellular carcinoma. *Hepatology* 1986; 6: 729–738.
3 The Liver Cancer Study Group of Japan. Primary liver cancer in Japan: Clinico-pathological features and results of surgical treatment. *Ann Surg* 1990; 211: 277–287.
4 Maynard JE. Hepatitis B: global importance and need for control. *Vaccine* 1990; 8: S18–S23.
5 Ikeda K, Saitoh S, Koida I *et al.* A multivariate analysis of risk factors for hepatocellular carcinogenesis – a prospective observation of 795 cases with viral and alcoholic cirrhosis. *Hepatology* 1993; 18: 47–53.
6 Tsukuma H, Hiyama T, Tanaka S *et al.* Risk factors for hepatocellular carcinoma among patients with chronic liver disease. *N Engl J Med* 1993; 328: 1797–1801.
7 Ikeda K, Saitoh S, Suzuki Y *et al.* Disease progression and hepatocellular carcinogenesis in patients with chronic viral hepatitis: a prospective observation of 2215 patients. *J Hepatol* 1998; 28: 930–938.
8 Prince AM, Szmuness W, Michon J *et al.* A case–control study of the association between primary liver cancer and hepatitis B infection in Senegal. *Int J Cancer* 1975; 16: 376–383.
9 Ohnishi K, Iida S, Iwama S *et al.* The effect of chronic habitual alcohols intake on the development of liver cirrhosis and hepatocellular carcinoma. Relation to hepatitis B surface antigen carriage. *Cancer* 1982; 49: 672–677.
10 Lam KC, Yu MC, Leung JWC, Henderson BE. Hepatitis B virus and cigarette smoking: Risk factors for hepatocellular carcinoma in Hong Kong. *Cancer Res* 1982; 42: 5246–5248.
11 Kaneko S, Muller RH, Feinstein SM *et al.* Detection of serum hepatitis B virus DNA in patients with chronic hepatitis using the polymerase chain reaction assay. *Proc Natl Acad Sci U S A* 1989; 86: 312–316.
12 Wang JT, Wang TH, Sheu JC, Shih LN, Lin JT, Chen DS. Detection of hepatitis B virus DNA by polymerase chain reaction in plasma of volunteer blood donors negative for hepatitis B surface antigen. *J Infect Dis* 1991; 163: 397–399.
13 Zhang YY, Hansson BG, Kuo LS, Widell A, Nordenfelt E. Hepatitis B virus DNA in serum and liver is commonly found in Chinese patients with chronic liver disease despite the presence of antibodies to HBsAg. *Hepatology* 1993; 17: 538–544.
14 Liang TJ, Baruch Y, Ben-Porath E *et al.* Hepatitis B virus infection in patients with idiopathic liver disease. *Hepatology* 1991; 13: 1044–1051.
15 Koike K, Kobayashi M, Gondo M, Hayashi I, Osuga T, Takada S. Hepatitis B virus DNA is frequently found in liver biopsy samples from hepatitis C virus-infected chronic hepatitis patients. *J Med Virol* 1998; 54: 249–255.
16 Cacciola I, Pollicino T, Squadrito G, Cerenzia G, Orlando ME, Raimondo G. Occult hepatitis B virus infection in patients with chronic hepatitis C liver disease. *N Engl J Med* 1999; 341: 22–26.
17 Takeuchi M, Fujimoto J, Niwamoto H, Yamamoto Y, Okamoto E. Frequent detection of hepatitis B virus X-gene DNA in hepatocellular carcinoma and adjacent liver tissue in hepatitis B surface antigen-negative patients. *Dig Dis Sci* 1997; 42: 2264–2269.
18 Chemin I, Zoulim F, Merle P *et al.* High incidence of hepatitis B infections among chronic hepatitis cases of unknown aetiology. *J Hepatol* 2001; 34: 447–454.
19 Sheu JC, Huang GT, Shih LN *et al.* Hepatitis C and B viruses in hepatitis B surface antigen-negative hepatocellular carcinoma. *Gastroenterology* 1992; 103: 1322–1327.
20 Paterlini P, Driss F, Pisi E, Franco D, Berthelot P, Brechot C. Persistence of hepatitis B and hepatitis C viral genomes in primary liver cancers from HBsAg-negative patients: a study of a low-endemic area. *Hepatology* 1993; 17: 20–29.
21 Lee DS, Huh K, Lee EH, Lee DH, Hong KS, Sung YC. HCV and HBV coexist in HBsAg-negative patients with HCV viraemia: possibility of co-infection in these patients must be considered in HBV-high endemic area. *J Gastroenterol Hepatol* 1997; 12: 855–861.
22 Brechot C, Jaffredo F, Lagorce D *et al.* Impact of HBV, HCV, and GBV-C/HGV on hepatocellular carcinomas in Europe: results of a European concerted action. *J Hepatol* 1998; 29: 173–183.
23 Shintani Y, Yotsuyanagi H, Moriya K *et al.* The significance of hepatitis B virus DNA detected in hepatocellular carcinoma of patients with hepatitis C. *Cancer* 2000; 88: 2478–2486.
24 Shiota G, Oyama K, Udagawa A *et al.* Occult hepatitis B virus infection in HBs antigen-negative hepatocellular carcinoma in a Japanese population: involvement of HBx and p53. *J Med Virol* 2000; 62: 151–158.
25 Tamori A, Nishiguchi S, Kubo S *et al.* HBV DNA integration and HBV-transcript expression in non-B, non-C hepatocellular carcinoma in Japan. *J Med Virol* 2003; 71: 492–498.
26 Pollicino T, Squadrito G, Cerenzia G *et al.* Hepatitis B virus maintains its pro-oncogenic properties in the case of occult HBV infection. *Gastroenterology* 2004; 126: 102–110.

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27 Yotsuyanagi H, Hashidume K, Suzuki M, Maeyama S, Takayama T, Uchikoshi T. Role of hepatitis B virus in hepatocarcinogenesis in alcoholics. *Alcohol Clin Exp Res* 2004; 28: 181S–185S.
28 Marusawa H, Osaki Y, Kimura T *et al.* High prevalence of anti-hepatitis B virus serological markers in patients with hepatitis C virus related chronic liver disease in Japan. *Gut* 1999; 45: 284–288.
29 Uetake S, Yamauchi M, Itoh S, Kawashima O, Takeda K, Ohata M. Analysis of risk factors for hepatocellular carcinoma in patients with HBs antigen- and anti-HCV antibody-negative alcoholic cirrhosis: clinical significance of prior hepatitis B virus infection. *Alcohol Clin Exp Res* 2003; 27(8 Suppl.): 47S–51S.
30 Yotsuyanagi H, Yasuda K, Iino S *et al.* Persistent viremia after recovery from self-limited acute hepatitis B. *Hepatology* 1998; 27: 1377–1382.
31 Kaplan EL, Meier P. Nonparametric estimation for incomplete observation. *J Am Stat Assoc* 1958; 53: 457–481.
32 Harrington DP, Fleming TR. A class of rank test procedures for censored survival data. *Biometrica* 1982; 69: 553–566.
33 Cox DR. Regression models and life tables. *J R Stat Soc* 1972; 34: 248–275.
34 SPSS Inc. SPSS for Windows Version 11.0 Manual. Chicago, IL, USA: SPSS Inc., 2001.
35 Beasley RP. Hepatitis B virus: the major etiology for hepatocellular carcinoma. *Cancer* 1988; 61: 1942–1956.
36 Matsubara K, Tokino T. Integration of hepatitis B virus may contribute to hepatocarcinogenesis. *Mol Biol Med* 1990; 7: 243–260.
37 Koshy R, Hofschneider PH. Transactivation by hepatitis B virus may contribute to hepatocarcinogenesis. *Curr Topics Microbiol Immunol* 1989; 144: 265–281.
38 Brechot C, Thiers V, Kremsdorf D, Nalpas B, Po S, Paterlini-Brechot P. Persistent hepatitis B virus infection in subjects without hepatitis B surface antigen: clinically significant or purely "occult"? *Hepatology* 2001; 34: 194–203.
39 Conjeevaram HS, Lok AS. Occult hepatitis B virus infection: a hidden menace? *Hepatology* 2001; 34: 204–206.
40 Hu KQ. Occult hepatitis B virus infection and its clinical implications. *J Viral Hepatitis* 2002; 9: 243–257.
41 Torbenson M, Thomas DL. Occult hepatitis B infection. *Lancet Infect Dis* 2003; 2: 479–486.
42 Brechot C, Degos F, Lugassy C *et al.* Hepatitis B virus DNA in patients with chronic liver disease and negative test for hepatitis B surface antigen. *N Engl J Med* 1985; 312: 270–276.
43 Kao JH, Chen PJ, Lai MY, Chen DS. Occult hepatitis B virus infection and clinical outcomes of patients with chronic hepatitis C. *J Clin Microbiol* 2002; 40: 4068–4071.
44 Guidotti LG, Chisari FV. To kill or to cure: options in host defense against viral infection. *Curr Opin Immunol* 1996; 8: 478–483.
45 Ikeda K, Kobayashi M, Someya T *et al.* Influence of hepatitis C virus subtype on hepatocellular carcinogenesis: a multivariate analysis of a retrospective cohort of 593 patients with cirrhosis. *Intervirology* 2002; 45: 71–78.
46 Marrero JA, Lok ASF. Occult hepatitis B virus infection in patients with hepatocellular carcinoma: innocent bystander, cofactor, or culprit? *Gastroenterology* 2004; 126: 347–350.
47 Castillo I, Pardo M, Bartolome J *et al.* Occult hepatitis C virus infection in patients in whom the etiology of persistently abnormal results of liver-function tests is unknown. *J Infect Dis* 2004; 189: 7–14.

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Original Article

Efficacy of platinum analogue for advanced hepatocellular carcinoma unresponsive to transcatheter arterial chemoembolization with epirubicin

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Aim: Hepatocellular carcinoma (HCC) often shows resistance to transcatheter arterial chemoembolization (TACE). Such patients often have a poor prognosis and are unresponsive to other forms of therapy. The aim of this retrospective study was to determine the response to TACE using platinum analogues in patients deemed resistant to TACE using epirubicin.

Methods: We studied 152 consecutive patients with advanced HCC resistant to TACE using epirubicin. All cases were treated with platinum analogue using transcatheter arterial chemotherapy with or without embolization.

Results: Computed tomography at 3 months after therapy showed complete response (CR) in 6 patients (4.0%), partial response (PR) in 28 (18%), stable disease (SD) in 35 (23%), and progressive disease (PD) in 83 (55%). The cumulative survival rates for PR/CR patients who received platinum analogue-transcatheter arterial chemotherapy with or without embolization (81.8% at first year, 53.9% at second year, and 33.1% at third year) were significantly higher than those of SD/PD patients (36.6%, 17.5% and 7.4%, respectively) ($P < 0.001$). The 50% survival period was extended almost 1.4 year in PR/CR patients who received platinum analogue-transcatheter arterial chemotherapy with or without embolization.

Conclusion: Our retrospective study is the first to report the efficacy of platinum analogues for advanced HCC unresponsive to TACE using epirubicin.

Key words: hepatocellular carcinoma, platinum analogue, transcatheter arterial chemoembolization, unresponsive

INTRODUCTION

HEPATOCELLULAR CARCINOMA (HCC) is one of the most common neoplasms in Africa and Asia including Japan. Since it is a well known that more than 80% of the cases with HCC are associated with liver cirrhosis, a routine check-up for cirrhotic patients with ultrasound (US) could potentially lead to the detection of small HCC. However, because of the association of cirrhosis and tumor multiplicity, surgical resection is performed only in 20% of the cases or less.[1,2] Transcatheter arterial chemoembolization (TACE) has been reported to be an effective palliative treatment for patients with unresectable HCC, and many chemotherapeutic agents such as doxorubicin, epirubicin, mitomycin were used with lipiodol in Japan.[3–10] Although repeated TACE is one of the most potent therapies for unresectable HCC, resistance to the therapy often ensues after therapy repetition, and long-term survival rates after 3 years are not sufficiently high at present.

Platinum analogues are effective against many malignant tumors, and in recent years, they have been used in the treatment of HCC. It has been reported that carboplatin-lipiodol treatment improved 1-year survival rate compared with doxorubicin-lipiodol treatment in patients with advanced HCC.[11] As for cisplatin, several studies reported its effectiveness for advanced HCC. Furthermore, the efficacy of cisplatin and lipiodol combination therapy has been reported by several investigators.[12–19] To our knowledge, however, there is no information on the efficacy of platinum analogues in TACE- epirubicin resistant HCC patients.

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The purpose of this retrospective study was to examine the efficacy of platinum analogues (carboplatin and cisplatin) for advanced HCC unresponsive to TACE using epirubicin.

PATIENTS AND METHODS

Study population

FROM 1980 TO 2006, 1,250 patients were diagnosed with HCC at the Department of Hepatology, Toranomon Hospital, Tokyo, Japan. Of these, 565 patients underwent TACE treatment for HCC. Among the 565 patients, 184 patients were judged by two hepatologists as embolization-resistant HCC and they received a platinum analogue. All 184 patients had been considered to have unresectable HCC at the time of diagnosis of HCC, and had undergone TACE therapy at least twice until being considered TACE-resistant. Embolization-resistant HCC was defined as an HCC whose number and/or size had increased in the treated segment and/or extended other segments despite repetitive course of TACE using epirubicin, lipiodol and gelatin sponge. Thus, this retrospective cohort study was based on 184 consecutive patients with TACE-resistant HCC.

Before treatment with carboplatin or cisplatin, all the patients underwent a comprehensive evaluation consisting of medical history, physical examination, measurement of tumor size, performance status, chest radiograph, liver imaging studies [computerized tomography (CT), ultrasonography (US), digital subtraction angiography (DSA)], complete blood count, and blood chemistry. Diagnosis of HCC was established based on the findings of US, CT, and DSA.

Of the 184 patients, 32 were excluded because they did not meet the following inclusion criteria: (i) typical hypervascular HCC by all imaging modalities; (ii) no history of other malignancies; (iii) no evidence of extrahepatic metastasis of HCC; (iv) performance status of 0–1; (v) adequate liver function with bilirubin value of 5 mg/dL or less; (vi) sufficient hematopoietic function with a platelet count of more than 25,000/mm^3 and leukocyte count of more than 2,000/mm^3; and (vii) an expected survival time of at least 3 months. All patients gave informed consent to the treatment. Accordingly, 152 patients with TACE-resistant HCC were retrospectively evaluated for efficacy of platinum analogue for advanced HCC unresponsive to TACE-epirubicin. The observation starting point was the time of first therapy using platinum analogue at our hospital.

Serologic markers for HCV and HBV

The diagnosis of HCV infection was based on detection of serum HCV antibody with RNA positivity. Anti-HCV was detected using a second-generation enzyme-linked immunosorbent assay (ELISA II) (Abbott Laboratories, North Chicago, IL). HCV-RNA was determined by the Amplicor method (Cobas Amplicor HCV Monitor Test, v2.0, Roche Molecular Systems, Inc., NJ). Hepatitis B surface antigen (HBs-Ag) was tested by radioimmunoassay (Abbott Laboratories, Detroit, MI). Serum HBV-DNA level was determined independently, using the nested PCR, by an experienced technician (M.K), who was blinded to the clinical information. The used serum samples were stored –80 °C at first consultation.

Treatment protocol

Patients were hydrated through a peripheral line. The femoral artery was catheterized under local anesthesia, and a catheter was inserted superselectively into the hepatic artery that supplied the target tumor, for injection of the platinum analogue with or without Lipiodol (Lipiodol Ultrafluide, Laboratoire Guerbet, Aulnay-sous-Bois, France) and small gelatin cubes (1 × 1 mm). The platinum analogue used was either carboplatin at 150 to 450 mg/body (63% of patients received 450 mg/body) or cisplatin at 40 to 100 mg/ body of (36% of patients received 100 mg/ body). Both analogues were administrated slowly under careful fluoroscopic guidance. When using Lipiodol, the platinum analogue and Lipiodol were divided into six to eight parts and mutually injected. In patients who received Lipiodol, the volume of injected Lipiodol ranged from 2.0 to 5.0 mL. The dose of Lipiodol was determined according to tumor size and the degree of liver dysfunction.

Selection criteria of type of therapy

Patients were treated by three type of therapy, depending on the extent of their tumors and liver function; (i)hepatic arterial injection (HAI) were performed for those patients with a tumor thrombus into main portal trunk or with severe liver function, (ii)chemolipiodalizatio (CL) were performed for those patients with tumor thrombus in distal portal branch complicated with severe liver function, (iii)TACE were performed for those patients without main portal vein thrombus and severe liver function.

Background and laboratory data

Table 1 summarizes the profiles and data of 152 patients who were treated with carboplatin or cisplatin. The

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Table 1 Demographics and laboratory data of 152 patients with HCC who underwent transcatheter arterial chemotherapy using platinum analogue for advanced HCC unresponsive to TACE-epirubicin

Parameter	(n = 152)
Patient characteristics	
Sex (M : F)	122:30
Age (years)	67 (38–85)
Back grounds of liver disease	
Hepatitis B surface antigen positive	27
HCV antibody positive	123
Both negative	2
Status of liver function	
Child–Pugh classification (A/B/C)	98/51/3
Laboratory data	
Platelet count ($\times 10^4$ /μL)*	11.0 (3.4–35.5)
Albumin (g/dL)*	3.0 (2.1–4.5)
Bilirubin (mg/dL)*	1.0 (0.3–4.3)
AST (IU/L)*	68 (21–488)
Prothrombin time (%)*	84 (45.5–114)
ICG R15 (%)*	38 (6.0–76)
AFP (μg/L)*	236 (2.0–112,000)
DCP (AU/L)*	153 (10–131,000)

*Expressed as median (minimum, maximum).
AFP, alpha-fetoprotein; AST, aspartate aminotransferase; DCP, des-gamma carboxyprothrombin; HCC, hepatocellular carcinoma; ICG R15, indocyanine green retention rate at 15 minutes; TACE, transcatheter arterial chemoembolization.

patients consisted of 122 men and 30 women, and their age ranged from 38 to 85 years (median, 67 years). They included 27 (18%) HBs-Ag positive patients, 123 (81%) HCV antibody positive patients, and 2 (1%) negative for both. At the time of the first platinum analogue treatment, the median serum albumin concentration was 3.0 g/dL, total bilirubin 1.0 mg/dL, indocyanine green retention rate at 15 minutes (ICG R15) 38%, prothrombin activity 84%, alpha-fetoprotein (AFP) 236 μg/L, and des-gamma-carboxyprothrombin (DCP) was 153 AU/L. As for Child-Pugh classification, 98 (64%) were class A, 51 (34%) were class B, and 3 (2%) were class C patients.

Characteristics of hepatocellular carcinoma

Table 2 summarizes the profiles of HCC that were treated with platinum analogue. The median tumor size was 40 mm. A solitary HCC was detected in 6 (4%) patients while multiple HCC were detected in 146 patients at the time of the first platinum analogue treatment. For the latter group, the tumors were localized to one segment in 10 (7%) patients, to one lobe in 32 (21%) patients, and in both lobes in 104 (68%) patients. Portal vein invasion was noted in 46 (30%) patients. The number of courses of TACE-epirubicin until judgment of embolization-resistance ranged from 2 to 16 with a median of 4 courses. The median interval between diagnosis of HCC and judgment of embolization-resistance was 30.1 months.

Table 2 Profile of HCC in 152 patients who underwent transcatheter arterial chemotherapy using a platinum analogue for advanced HCC unresponsive to TACE-epirubicin

Profiles of liver cancer	
Tumor size (mm)*	40 (8–180)
Intrahepatic multiplicity	
Solitary	6
Multiple, localized to one segment	10
Multiple, localized to one lobe	32
Multiple, extended to both lobes	104
Portal vein invasion (no/yes)	106/46
Embolization iteration until unresponsiveness	4 (2–16)
The kind of used platinum analogue	
Carboplatin/Cisplatin	105/47
Treatment method	
HAI/CL/TACE	73/20/59

*Expressed as median (minimum, maximum).
CL, chemolipiodalization; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

The type of platinum analogue used for treatment was carboplatin for 105 (69%) patients, and cisplatin for 47 (31%) patients. With regard to the method used for delivery of platinum analogue, hepatic arterial injection (HAI) was used in 73 (48%) patients, chemolipiodalization (CL) was used in 20 (13%) patients and TACE in 59 (39%) patients.

Assessment of therapeutic effects and follow-up

The effects of chemotherapy were evaluated by CT every three months after treatment. The presence of non-enhanced tumor areas reflects tissue necrosis, and according to the findings of this imaging technique, the response to treatment was defined according to the World Health Organization criteria:[20] complete response: no evidence of neoplastic disease; partial response: reduction in total tumor load of ≥ 50%; no change: reduction of < 50% or increase of < 25%; progressive disease: increase of ≥ 25%.

Patients were examined by physicians every 4 weeks including monitoring of AFP, DCP and other biochemical data after the diagnosis of embolization-resistance.

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Table 3 Profile of 152 HCC patients who underwent transcatheter arterial chemotherapy using a platinum analogue for advanced HCC unresponsive to TACE-epirubicin, according to type of therapy

	HAI ($n = 73$)	CL ($n = 20$)	TACE ($n = 59$)
Profile of liver cancer and tumor marker			
Tumor size (mm)*	48 (8–180)	40 (8–100)	33 (12–180)
Intrahepatic multiplicity			
Solitary	4	1	1
Multiple, localized to one segment	3	2	5
Multiple, localized to one lobe	14	7	11
Multiple, extended to both lobes	52	10	42
Portal vein invasion (no/yes)	45/28	12/8	49/10
AFP (μg/L)*	257 (3–112000)	682 (17–65900)	145 (2–103000)
DCP (AU/L)*	400 (10–131000)	68 (10–53900)	40 (10–55420)
Status of liver function			
Child-Pugh classification (A/B/C)	43/28/2	13/6/1	42/17/0

*Expressed as median (minimum, maximum).
AFP, alpha-fetoprotein; CL, chemolipiodalization; DCP, des-gamma carboxyprothrombin; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

Imaging studies, as required for measurement of tumor size, were performed at around 3 months after therapy. Some patients took oral or intravenous medicinal herbs or other palliative therapies during the follow-up period.

Statistical analysis and ethical considerations

The factors that influenced the treatment outcome ((partial response (PR) or complete response (CR)) in this cohort were analyzed by the χ^2 test, and the cumulative survival rate was analyzed by Kaplan-Meier method. The risk factors involved in survival were evaluated by univariate analysis with the log-rank test. The independent factors associated with the curative effect (PR or CR) and survival rate were identified using the stepwise Cox regression analysis. Potential risk factors assessed for curative outcome (PR and CR) and survival rate included the following 17 variables: age, sex, HBs-Ag, HCV-antibody, aspartate transaminase (AST), albumin, bilirubin, AFP, DCP, prothrombin activity, ICG-R15, tumor size, multiplicity, portal vein invasion of HCC, treatment methods (HAI/CL/TACE), the type of platinum analogue (carboplatin/cisplatin) and the dose of platinum analogue used for treatment. Several variables were transformed into categorical data consisting of two-three simple ordinal numbers for univariate and multivariate analyses. All factors found to be at least marginally associated with the curative effect and survival ($P < 0.10$) in univariate analysis were entered into a multivariate logistic regression and Cox proportional hazard models. Significant variables were selected by stepwise method in the procedure. Proportional hazard analysis was also employed in the identification of contributing factors to the curative effect and survival rate. A P-value of less than 0.05 in two-tailed test was considered significant. Data analysis was performed using SPSS software version 11.0 (SPSS Inc, Chicago, Ill).

The study protocol was approved by the Human Ethics Review Committee of Toranomon Hospital. The physicians in charge explained the purpose and method of the clinical trial to each patient, who gave their informed consents for participation.

RESULTS

Efficacy of platinum analogue, according to type of therapy

TABLE 3 SUMMARIZES the profiles and data of 152 HCC patients who were treated with platinum analogue, according to type of therapy.

In these patients, 6 of 152 (4%) patients showed CR, 28 (18%) patients showed PR, 35 (23%) patients showed stable disease (SD), and 83 (55%) patients showed progressive disease (PD). Analysis according to type of therapy showed 73 of 152 (48%) patients received HAI, 20 (13%) received CL, and 59 (39%) received TACE. The efficacy of transcatheter arterial chemotherapy using platinum analogue according to the type of therapy was as follow; in HAI group: 1 of 73

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Table 4 Efficacy of transcatheter arterial chemotherapy using platinum analogue for advanced HCC in 152 patients unresponsive to TACE-epirubicin, according to type of therapy

	CR (%)	PR (%)	SD (%)	PD (%)
Total ($n = 152$)	6 (4%)	28 (18%)	35 (23%)	83 (55%)
Type of therapy				
HAI ($n = 73$)	1 (1%)	9 (12%)	16 (22%)	47 (65%)
CL ($n = 20$)	1 (5%)	3 (15%)	7 (35%)	9 (45%)
TACE ($n = 59$)	4 (7%)	16 (27%)	12 (20%)	27 (46%)

CL, chemolipiodalization; CR, complete remission; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; PD, progressive disease; PR, partial response; SD, stable disease; TACE, transcatheter arterial chemoembolization.

(1%) patients showed CR, 9 of 73 (12%) patients showed PR, 16 of 73 (22%) patients showed SD, and 47 of 73 (65%) patients showed PD; in CL group: 1 of 20 (5%) patients showed CR, 3 of 20 (15%) patients showed PR, 7 of 20 (35%) patients showed SD, and 9 of 20 (45%) patients showed PD; in TACE group: 4 of 59 (7%) patients showed CR, 16 of 59 (27%) patients showed PR, 12 of 59 (20%) patients showed SD, and 27 of 59 (46%) patients showed PD (Table 4).

Factor influencing curative effect (PR or CR)

We then investigated the factors associated with curative effect (PR or CR) after treatment using platinum analogue. Univariate analysis identified the following 11 factors that influenced the rate of curative effect (PR or CR): serum DCP (< 100 IU/L/≥ 100 IU/L, $P = 0.001$), serum AFP (< 200 μg/L/≥ 200 μg/L, $P = 0.005$), ICG-R15 (< 30%/≥ 30%, $P = 0.005$), tumor size (< 20 mm/ ≥ 20 mm, $P = 0.011$), portal vein invasion (yes/no, $P = 0.014$), total bilirubin (< 1.5 mg/dL/≥ 1.5 mg/dL, $P = 0.018$), treatment method (HAI/CL/TACE, $P = 0.021$), type of platinum analogue (carboplatin/cisplatin, $P = 0.021$), intrahepatic multiplicity that extended to both lobes (yes/no, $P = 0.028$), age (< 60/ ≥ 60, $P = 0.057$), and serum AST (< 50 IU/L/dL/ ≥ 50 IU/L, $P = 0.057$). These parameters were entered into multivariate logistic regression analysis. The curative effect (PR or CR) was significantly higher for elderly patients (aged ≥ 60, risk ratio: 7.75; 95% CI: 1.80–33.40), small size HCC (< 20 mm, risk ratio: 4.88; 95% CI: 1.62–14.71), TACE-platinum analogue treatment (yes, risk ratio: 3.91; 95% CI: 1.34–11.38), lower serum total bilirubin level (< 1.5 mg/dL, risk ratio: 3.44; 95% CI: 1.22–9.71), and Tumor multiplicity, extended to both lobes (no, risk ratio: 2.30 (1.03–7.09) (Table 5).

Table 5 Factors associated with curative effects in patients who underwent transcatheter arterial platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin

Factors	Category	Risk Ratio (95% confidence interval)	*P*
Age (year)	1: < 60	1	
	2: ≥ 60	7.75 (1.80–33.40)	0.006
Tumor size (mm)	1: ≥ 20	1	
	2: < 20	4.88 (1.62–14.71)	0.005
Tumor therapy	1: HAI	1	
	2: CL	2.47 (0.52–11.69)	0.256
	3: TACE	3.91 (1.34–11.38)	0.012
Bilirubin (mg/dL)	1: ≥ 1.5	1	
	2: < 1.5	3.44 (1.22–9.71)	0.020
Multiple HCC, extended to both lobes (yes/no)	1: yes	1	
	2: no	2.30 (1.03–7.09)	0.044

CL, chemolipiodalization; CR, complete remission; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; PR, partial response; TACE, transcatheter arterial chemoembolization.

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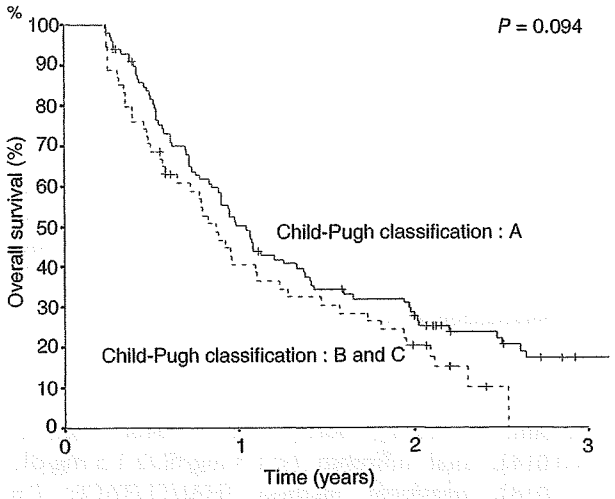

Figure 1 Cumulative survival rates after the first use of platinum-analogue for advanced HCC unresponsive to TACE-epirubicin, according to Child-Pugh classification.

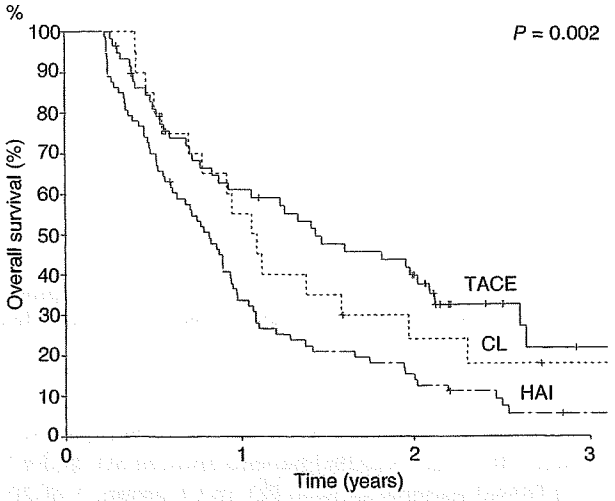

Figure 2 Cumulative survival rates after the first use of platinum-analogue for advanced HCC unresponsive to TACE-epirubicin, according to type of therapy.

Cumulative survival rate according to Child-Pugh classification

During the observation period, 125 of 152 (82.2%) patients died. The cumulative survival rates after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin according to Child-Pugh classification were 50.1% at the end of the first year, 28.7% at the second year, and 17.2% at the third year for patients with Child-Pugh class A, and 40.5% at the first year, 20.3% at the second year, 0% at the third year for patients with Child-Pugh class B and C. The cumulative survival rates were slightly higher in patients with Child-Pugh class A than in those with Child-Pugh class B and C ($P = 0.094$), but no statistical significance was not there (Fig. 1).

Cumulative survival rate according to type of therapy

The cumulative survival rates after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin according to type of therapy were 33.6% at the end of the first year, 15.4% at the second year, and 5.6% at the third year for patients with HAI group, 55.0% at the first year, 24.0% at the second year, 18.0% at the third year for patients with CL group, and 60.8% at the first year, 40.0% at the second year, 21.7% at the third year for patients with TACE group.

The cumulative survival rate was significantly different in these three type of therapy ($P = 0.002$) (Fig. 2).

Cumulative survival rate according to treatment effect

The cumulative survival rates after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin were 81.8% at the end of the first year, 53.9% at the second year, and 33.1% at the third year for patients with PR or CR, and 36.6% at the first year, 17.5% at the second year, 7.4% at the third year for patients with PD or SD. The cumulative survival rates were significantly higher in patients with PR or CR than in those with SD or PD by transcatheter arterial chemotherapy using platinum analogue ($P < 0.001$) (Fig. 3). The 50% survival period was extended almost 1.4 year in patients with PR or CR by transcatheter arterial chemotherapy using platinum analogue.

Factor affecting survival rate

We then investigated the factors associated with survival rate after the first transcatheter arterial chemotherapy using platinum analogue for advanced HCC unresponsive to TACE-epirubicin. Univariate analysis identified the following 13 factors that influenced the survival rate: portal vein invasion (yes/no, $P < 0.001$), type of platinum analogue (carboplatin/cisplatin, $P = 0.001$), tumor size (< 20 mm/≥ 20 mm, $P = 0.001$), serum DCP (< 100 IU/L/≥ 100 IU/L, $P = 0.001$), age (< 60/≥ 60, $P = 0.001$), serum AFP (< 200 μg/L/≥ 200 μg/L, $P = 0.006$), treatment method (HAI/CL/TACE, $P = 0.008$), intrahepatic multiplicity that extended to both lobes

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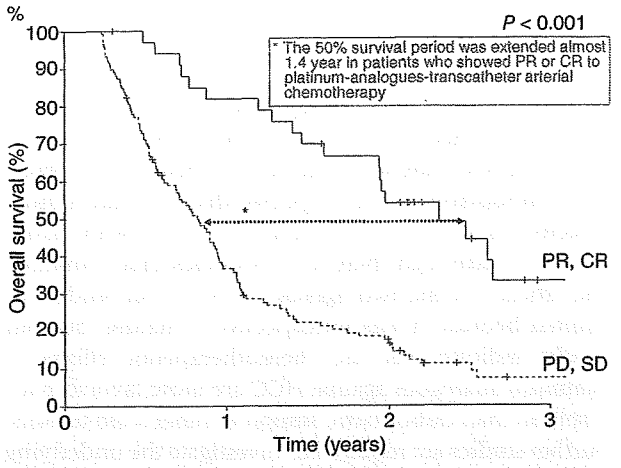

Figure 3 Overall survival rates after the first use of platinum-analogue for advanced HCC unresponsive to TACE-epirubicin, according to treatment effect.

(yes/no, $P = 0.012$), serum AST (< 50 IU/L/dL/ ≥ 50 IU/L, $P = 0.014$), prothrombin activity (< 80%/ ≥ 80%, $P = 0.020$), HBs-Ag (positive/negative, $P = 0.048$), anti HCV antibody (positive/negative, $P = 0.063$), and total bilirubin (< 1.5 mg/dL/≥ 1.5 mg/dL, $P = 0.068$). These parameters were entered into multivariate Cox proportional hazard analysis. The curative survival rate was significantly higher for small size HCC (< 20 mm, hazard ratio: 2.60; 95% CI: 1.30–5.19), no evidence of portal vein invasion (hazard ration: 2.08; 95% CI: 1.37–3.16), TACE treatment (yes, hazard ratio: 1.93; 95% CI: 1.23–3.02), HBs antigen negative (yes, hazard ratio: 1.82; 95% CI: 1.13–2.93), and low AST level (< 50 IU/L, hazard ratio: 1.67; 95% CI: 1.06–2.61) (Table 6).

Toxic effects

The most common side effects observed after treatment with a platinum analogue were fever and vomiting. Low-grade fever of 37–38°C lasting for a few days occurred in 78 (51.3%) patients, and fever of ≥ 38°C occurred in the other 51 (33.6%) patients. Nausea was reported in 125 (82.2%) patients, vomiting was noted at the time of treatment in 49 (32.2%) patients, dull pain in the upper abdomen was reported at the time of infusion by 31 patients (20.4%), a rise in serum aminotransferases was seen in 80 (52.6%) patients: 36 showed a rise up to twice the values before treatment, and 44 showed a rise of 1.5 to 1.9 times the baseline values. Within one week of treatment, 42 (27.6%) patients showed a transient rise in total bilirubin level to twice the pretreatment value. No adverse effects due to embolization in critical organs such as the lung, the heart, or brain were noted.

Causes of death

During the observation period, 125 (82.2%) patients died. The cause of death was HCC in 106 (84.8%) patients, liver failure in 14 (11.2%) patients and other causes in 5 (4.0%) patients.

Table 6 Factors associated with overall survival rate in patients with underwent transcatheter arterial platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin (Multivariate Cox proportional hazard analysis)

Factors	Category	Hazard Ratio (95% confidence interval)	*P*
Tumor size (mm)	1: ≥ 20	1	
	2: < 20	2.60 (1.30–5.19)	0.007
Portal vein invasion (yes/no)	1: yes	1	
	2: no	2.08 (1.37–3.16)	0.001
Tumor therapy	1: HAI	1	
	2: CL	1.80 (1.00–3.24)	0.050
	3: TACE	1.93 (1.23–3.02)	0.004
HBs antigen	1: positive	1	
	2: negative	1.82 (1.13–2.93)	0.013
AST (IU/L)	1: ≥ 50	1	
	2: < 50	1.67 (1.06–2.61)	0.026

CL, chemolipiodalization; HAI, hepatic arterial injection; HCC, hepatocellular carcinoma; TACE, transcatheter arterial chemoembolization.

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DISCUSSION

ALTHOUGH TACE IS one of the most potent methods of treatment for unresectable HCC, various types of resistances to therapy can occur during the repetition of embolization. However, to our knowledge, there is no report that assessed the efficacy of platinum analogue for TACE-resistant HCC patients. Since various chemotherapeutic agents have occasionally produced objective tumor regression in palliative management of advanced liver cancer, we used platinum analogues for embolization-resistant HCC.

In our study, the 50% survival period was extended almost 1.4 year in patients who showed PR or CR in response to transcatheter arterial chemotherapy using platinum analogue for TACE-resistant HCC. We consider this outcome encouraging in TACE-resistant HCC, because these types of tumors are usually unexpected respond to any treatment. Multivariate analysis indicated that it is important to deliver the platinum analogue via TACE in order to achieve a satisfactory curative effect. A previous study reported the efficacy of cisplatin when injected with Lipiodol for preoperative TACE therapy (termed "sandwich therapy") in patients with HCC who received TACE as the first treatment.[14]

In univariate analysis, the cumulative survival rates were slightly higher in patients with Child-Pugh class A than in those with Child-Pugh class B and C, but in multivariate analysis, the factor of liver function was not significantly related to survival rate. In the result of multivariate analysis, tumor related factors more affected on the cumulative survival rates than liver function in this study.

The type of therapy, in univariate analysis, the cumulative survival rates were significantly different among the therapies. Multivariate analysis disclosed that the type of therapy significantly affected on cumulative survival rate after the first use of platinum analogue therapy for advanced HCC unresponsive to TACE-epirubicin.

These results indicated that it is important to deliver the platinum analogue via TACE in order to obtain a more long term survival, as well as curative effect.

Our results showed that the survival rate of HBV-positive HCC patients was significantly lower than that of HCV-positive patients with advanced HCC. HBV-related HCC or non-viral hepatitis-related HCC are often diagnosed at a more advanced stage than HCV-related HCC, and patients with such advanced-stage HCC related to HBV or non-viral etiology showed poor prognosis compared to those with HCV-related HCC.[21] This may explain the lower survival rate in our patients with HBV-related HCC.

In this study, we used two types of platinum analogues (cisplatin and carboplatin), and the results of univariate analysis, the curative effect (PR and CR) and overall survival rate were significantly better for cisplatin than carboplatin use. It is reported that cisplatin is more effective as an anti-tumor agent compared with carboplatin,[22,23] although there are no studies that compared the efficacy of the two agents in HCC. Our study was limited because it was retrospective in nature, but our results indicate that the chemotherapeutic effects of platinum analogues against HCC are more favorable for cisplatin than carboplatin, similar to other solid tumors. Further studies are required to investigate the underlying mechanism for the difference in efficacy of cisplatin than carboplatin.

A number of molecular-based chemotherapeutic agents are expected to become available in the future, such as Sorafenib,[24] and the primary therapy of advanced stage HCC may change with the introduction of these drugs. However, the results of our study suggested the advantage of using cisplatin in patients with TACE-resistant HCC. Although further studies are required to confirm our findings, the combination of various types of molecular targeting drugs and TACE may improve the treatment outcome in advanced-stage HCC.

In conclusion, the present study reports the efficacy of platinum analogues in patients with advanced HCC unresponsive to TACE-epirubicin. Most such patients have poor prognosis mainly because of lack of effective therapy. However, our results show that the 50% survival period of patients who respond to platinum analogues-transcatheter arterial chemotherapy was extended to almost 1.4 years. Accordingly, we recommend this form of chemotherapy for patients with advanced HCC unresponsive to TACE-epirubicin.

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REFERENCES

1 The Liver Cancer Study Group of Japan. Primary liver cancer in Japan. *Cancer* 1987; 60: 1400–11.

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2 The Liver Cancer Study Group of Japan. Primary liver cancer in Japan: clinicopathological features and results of surgical treatment. *Ann Surg* 1990; **211**: 277–87.
3 Wheeler PG, Melia W, Dubbins P *et al.* Non-operative arterial embolization in primary liver tumours. *Br Med J* 1979; 2: 242–4.
4 Chuang VP, Wallace S. Hepatic arterial embolization in the treatment of hepatic neoplasms. *Radiology* 1981; **140**: 51–8.
5 Okamura J, Horikawa S, Fujiyama T *et al.* An appraisal of transcatheter arterial infusion of chemotherapeutic agent for hepatic malignancies. *World J Surg* 1982; 6: 352–7.
6 Yamada R, Sato M, Kawabata M, Nakatsuka H, Nakamura K, Takashima S. Hepatic artery embolization in 120 patients with unresectable hepatoma. *Radiology* 1983; **148**: 397–401.
7 Lin DY, Liaw YF, Lee TY, Lai CM. Hepatic arterial embolization in patients with unresectable hepatocellular carcinoma: a randomized controlled trial. *Gastroenterology* 1988; **94**: 453–6.
8 Ikeda K, Kumada H, Saitoh S, Arase Y, Chayama K. Effect of repeated transcatheter arterial embolization on the survival time in patients with hepatocellular carcinoma. *Cancer* 1991; **68**: 2150–4.
9 Llovet JM, Real MI, Montaña X *et al.* Barcelona Liver Cancer Group. Arterial embolisation or chemoembolisation versus symptomatic treatment in patients with unresectable hepatocellular carcinoma: a randomised controlled trial. *Lancet* 2002; **359**: 1734–9.
10 Lo CM, Ngan H, Tso WK *et al.* Randomized controlled trial of transarterial lipiodol chemoembolization for unresectable hepatocellular carcinoma. *Hepatology* 2002; **35**: 1164–71.
11 Homma H, Mezawa S, Doi T *et al.* A comparative randomized trial of intermittent intrahepatic arterial carboplatin-versus doxorubicin-lipiodol emulsion in advanced hepatocellular carcinoma (stage IV). *Hepatogastroenterology* 2004; **51**: 1135–9.
12 Shibata J, Fujiyama S, Sato T, Kishimoto S, Fukushima S, Nakano M. Hepatic arterial injection chemotherapy with cisplatin suspended in an oily lymphographic agent for hepatocellular carcinoma. *Cancer* 1989; **64**: 1586–94.
13 Sasaki Y, Imaoka S, Kasugai H *et al.* A new approach to chemoembolization therapy for hepatoma using ethiodized oil, cisplatin, and gelatin sponge. *Cancer* 1987; **60**: 1194–203.
14 Imaoka S, Sasaki Y, Shibata T *et al.* A pre-operative chemoembolization therapy using lipiodol, cisplatin and gelatin sponge for hepatocellular carcinoma. *Cancer Chemother Pharmacol* 1989; 23: S126–8.
15 Yamamoto K, Shimizu T, Narabayashi I. Intraarterial infusion chemotherapy with lipiodol-CDDP suspension for hepatocellular carcinoma. *Cardiovasc Intervent Radiol* 2000; **23**: 26–39.
16 Maeda S, Shibata J, Fujiyama S *et al.* Long-term follow-up of hepatic arterial chemoembolization with cisplatin suspended in iodized oil for hepatocellular carcinoma. *Hepatogastroenterology* 2003; **50**: 809–13.
17 Ikeda M, Maeda S, Shibata J *et al.* Transcatheter arterial chemotherapy with and without embolization in patients with hepatocellular carcinoma. *Oncology* 2004; **66**: 24–31.
18 Ando E, Tanaka M, Yamashita F *et al.* Hepatic arterial infusion chemotherapy for advanced hepatocellular carcinoma with portal vein tumor thrombosis: analysis of 48 cases. *Cancer* 2002; **95**: 588–95.
19 Kaneko S, Urabe T, Kobayashi K. Combination chemotherapy for advanced hepatocellular carcinoma complicated by major portal vein thrombosis. *Oncology* 2002; **62**: 69–73.
20 Miller AB, Hoogstraten B, Staquet M, Winkler A. Reporting results of cancer treatment. *Cancer* 1981; **47**: 207–14.
21 Hatanaka K, Kudo M, Fukunaga T *et al.* Clinical characteristics of NonBNonC- HCC: comparison with HBV and HCV related HCC. *Intervirology* 2007; **50**: 24–31.
22 Lokich J, Anderson N. Carboplatin versus cisplatin in solid tumors: an analysis of the literature. *Ann Oncol* 1998; 9: 13–21. Review.
23 Go RS, Adjei AA. Review of the comparative pharmacology and clinical activity of cisplatin and carboplatin. *J Clin Oncol* 1999; **17**: 409–22. Review.
24 Llovet JM, Bruix J. Novel advancements in the management of hepatocellular carcinoma in 2008. *J Hepatol* 2008; 48 (Suppl 1): S20–37. Review.

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High Serum Des-gamma-carboxy Prothrombin Level Predicts Poor Prognosis After Radiofrequency Ablation of Hepatocellular Carcinoma

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BACKGROUND: Currently, surgical resection is considered the first-line treatment for early stage hepatocellular carcinoma (HCC). Radiofrequency ablation (RFA) has been an alternative choice for unresectable HCC. However, RFA is expected to have similar therapeutic efficacy for early stage HCC with fewer invasions. **METHODS:** The authors retrospectively analyzed 199 patients who underwent surgery and 209 patients who underwent RFA for HCC with a maximum diameter of ≤3 cm and tumors numbering ≤3. All patients were complicated with Child-Pugh A cirrhosis. **RESULTS:** The 3- and 5-year survival rates of the resection (90.3%, 79.0%, respectively) and RFA groups were similar (87.4%, 74.8). The 1- and 3-year tumor recurrence-free survival rates of the resection group (83.1%, 51.0%, respectively) were higher than in the RFA group (82.7%, 41.8%; $P=.011$). Multivariate analysis identified prothrombin time ≥80% (hazard ratio [HR], 2.72; 95% confidence interval [CI], 1.56-4.74; $P < .001$) as an independent prognostic factor for survival in the resection group. Des-gamma-carboxy prothrombin (DCP) <100 arbitrary units (AU)/L (HR, 5.49; CI, 2.23-13.5; $P < .001$) and platelet count $\geq 1.0 \times 10^5$ (HR, 2.70; CI, 1.24-5.88; $P=.012$) were significant markers in the RFA group. Among patients with DCP ≥100 AU/L, treatment procedure (HR, 1.26; CI, 1.04-1.53; $P=.020$) was a significant prognostic factor for survival. **CONCLUSIONS:** High DCP levels reflect the biologic aggressiveness and progression of HCC tumors. In the aforementioned cases, we recommend surgical resection rather than RFA for such patients. **Cancer 2009;115:571–80. © 2008 American Cancer Society.**

KEY WORDS: hepatocellular carcinoma, DCP, radiofrequency, prognostic factor.

Hepatocellular carcinoma (HCC) is the sixth most common malignancy and third most deadly carcinoma in the world.[1] In Japan, HCC is ranked third among males and fifth among females as the leading causes of cancer death.[2] Most patients with HCC are infected with either hepatitis B virus (HBV) or hepatitis C virus (HCV), and have complications stemming from underlying chronic liver disease. The importance of liver condition in the treatment of HCC should be clearly discerned.[3]

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A maximum tumor diameter of ≤3 cm and tumors numbering ≤3 are good candidates for liver transplantation in patients with Child-Pugh class B and C.[4,5] However, patients with Child-Pugh class A conditions should be treated curatively.[5,6] Hepatectomy is currently recommended for patients with single asymptomatic HCC and extremely well-preserved liver function, who have neither clinically substantial portal hypertension nor abnormal bilirubin levels.[6] However, resection is suitable for only 20% to 35% of patients with HCC because of poor hepatic reserve.[7,8] Radiofrequency ablation (RFA) was introduced as a minimally invasive therapy for such cirrhotic patients.[9-13] RFA was the initial choice for unresectable HCC; however, 2 recent randomized controlled trials concluded that there were no substantial statistical survival differences between resection and RFA.[9,10] Although the results of these studies have not yet reached a worldwide consensus, some authors recommend RFA as a first-line therapy for such early stage HCC.[11-13]

Tumor staging and the decision between possible treatment options are conducted predominantly based on tumor size, number, vascular invasion, and extrahepatic metastasis evaluated by imaging analysis such as ultrasonography or dynamic computed tomography (CT). However, the malignant nature of the tumor as well as other characteristics are not generally considered.[14,15] Alpha-fetoprotein (AFP) and des-gamma-carboxy prothrombin (DCP) are HCC-specific tumor markers. High levels of serum tumor markers often indicate HCC development in the liver. On the basis of histopathological analysis, serum AFP and DCP levels are also correlated with tumor differentiation, microscopic portal invasion, or intrahepatic metastasis.[16,17]

This present study is an attempt to evoke discussion on treatment strategies for small HCC measuring ≤3 cm by comparing the long-term outcome of patients treated with either hepatectomy or RFA as the first-line treatment for HCC. AFP and DCP were also accounted as indicators in the decision-making and treatment procedure.

MATERIALS AND METHODS

Patients

A total of 1057 patients were admitted to the Department of Hepatology, Toranomon Hospital between 1995 and 2006 for the treatment of initially developed HCC. The major background liver disease was HCV (767 patients, 72.6%), followed by HBV (196 patients, 18.5%), HCV + HBV (8 patients, 0.8%), alcoholic liver diseases (habitual drinking of ethanol at >80 g/day, 48 patients, 4.5%), primary biliary cirrhosis (4 patients, 0.4%), autoimmune hepatitis (2 patients, 0.2%), and cryptogenic liver disease (42 patients, 4.0%). Treatment of HCC included surgical resection in 281 patients, local ablation therapy in 398 patients (RFA, 267 patients; microwave coagulation, 47 patients; ethanol injection, 84 patients), and transarterial chemoembolization in 378 patients. Among these patients, we included patients with Child-Pugh A cirrhosis and HCC measuring ≤3 cm in diameter and numbering ≤3 tumors who were treated radically by either surgical resection or RFA. Table 1 summarizes the profile of the 199 patients who received resection and 209 patients who received RFA. HBV-related liver diseases were more common among patients who underwent resection, who were younger (62 vs 67 years; $P < .001$) than patients with RFA. The maximum tumor diameter was larger in the resection group than in the RFA group (20 vs 18 mm; $P < .001$). With regard to laboratory tests, serum albumin level, platelet count, and prothrombin time (%) were higher among patients in the resection group, whereas serum aspartate aminotransferase (AST) levels were higher among patients in the RFA group. None of the patients in either group had tumor invasion of the major portal branch or extrahepatic metastasis. Our institution does not require informed consent for retrospective analysis.

Diagnosis of HCC

Diagnosis of HCC was predominantly based on image analysis. If a hepatic nodular lesion was found on screening ultrasonography, the patient underwent dynamic CT and/or dynamic magnetic resonance imaging (MRI). Furthermore, when a liver nodule showed hyperattenuation in the arterial phase of dynamic study and washout in portal or delayed phase, or showed typical hypervascular staining on digital subtraction angiography, the nodule was diagnosed as HCC. According to the American Association for the Study of Liver Disease guidelines, we obtained at least 2 dynamic imaging images before treatment.[5] When the nodule did not appear in the abovementioned typical imaging features, a fine needle aspiration biopsy was carried out followed by histological examination and diagnosis.