



ORIGINAL ARTICLE

## Potential risk factors for the reactivation of the replication of hepatitis B and C viruses after transcatheter arterial chemoembolization of hepatocellular carcinoma

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Received 28 January 2011; accepted 16 May 2011

Available online 25 November 2011

### KEYWORDS

Hepatitis B virus;  
Hepatitis C virus;  
Hepatocellular carcinoma;  
Transcatheter arterial chemoembolization;  
White blood cell

**Abstract** The purpose of this study was to investigate the potential risk factors for the reactivation of the replication of hepatitis B virus (HBV) and hepatitis C virus (HCV) after transcatheter arterial chemoembolization (TACE) of hepatocellular carcinoma. Forty-four hepatocellular carcinoma patients treated by TACE using epirubicin plus mitomycin C were studied. Serum HBV DNA ( $n = 17$ ) and HCV RNA ( $n = 27$ ) levels were measured 1 day before and 3 months after TACE. Plasma concentrations of chemotherapeutic agents were determined at 1 hour and 72 hours after TACE. A total of 29 patients ( $n = 13$  for chronic hepatitis B and  $n = 16$  for chronic hepatitis C) showed significant changes of the viral loads after TACE. Patients with increased viral loads after TACE were older ( $p = 0.041$ ), had higher incidence of pre-TACE white blood cell counts being less than normal limit ( $p = 0.023$ ), and had higher plasma mitomycin C concentrations ( $p = 0.039$ ) than those in patients with decreased viral loads. Analysis by multiple logistic regressions using age, decreased or normal pre-TACE white blood cell counts, mitomycin C concentrations  $>3.95$  ng/mL adopted by receiver operating characteristic curve ( $p = 0.037$ ), and epirubicin concentrations have shown that decreased

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pre-TACE white blood cell counts was the only significant factor associated with increased viral loads after TACE ( $p = 0.048$ ). In conclusion, patients with decreased pre-TACE white blood cell counts have a potential risk for the reactivation of the replication of HBV or HCV after TACE. Copyright © 2011, Elsevier Taiwan LLC. All rights reserved.

## Introduction

Hepatocellular carcinoma (HCC) is the third most common cause of cancer-related deaths worldwide [1]. Chronic hepatitis B virus (HBV) and hepatitis C virus (HCV) infections comprise the major etiologies in the development of HCC [1,2]. Transcatheter arterial chemoembolization (TACE) is one of the palliative treatments in the management of HCC [2–5]. Our previous study showed that a high proportion of HCC patients treated by TACE had detectable plasma chemotherapeutic concentrations within 72 hours after the procedure [6]. Reactivation of HBV or HCV is frequently observed in patients after immunosuppressive therapy [7–13]. However, influence of TACE on viral replication remains controversial [14–17], which might partly be attributed to differences in the study designs and selections of patients and chemotherapeutic agents. The association between the plasma concentrations of chemotherapeutic agents and the reactivation of viral replication after TACE has rarely been investigated. The purpose of this prospective study was to investigate the potential risk factors for the reactivation of replication of HBV and HCV after TACE. The concentrations of leaked chemotherapeutic agent after TACE were added in the analysis.

## Materials and methods

### Patient selection

From February 2008 to February 2009, a total of 44 consecutive HBV- or HCV-infected HCC patients who planned to receive TACE and agreed to participate this study were enrolled (Table 1). Patients who had been treated by antiviral therapy for chronic hepatitis B (CHB) or chronic hepatitis C (CHC) were excluded. None of patients received

antiviral therapy during this study period. The diagnosis of HCC was based on fine-needle aspiration cytology and/or biopsy with positive findings on computed tomography and angiography. HCC staging was based on the American Joint Committee on Cancer TNM staging system [18]. Patients with large arterioportal shunt, which needed to be treated by implantation of various sizes of Nester Embolization Coils (Cook Incorporated, Bloomington, IN, USA), were excluded. Patients with chronic CHB or CHC were diagnosed by seropositivity of hepatitis B surface antigen or anti-HCV antibody for more than 6 months. Child-Pugh classification was applied to evaluate the hepatic reserved function. The study was approved by the ethics committees at the participating hospital and was carried out according to the guidelines of the International Conference on Harmonization for Good Clinical Practice. All patients gave written informed consent before enrollment.

### Procedure of TACE

TACE was carried out by infusion of the mixture of chemotherapeutic agents and lipiodol (Lipiodol Ultra-Fluid; Guerbet, Aulnay-sous-Bois, France) into the lesion followed by embolization of the supplying arteries using various sizes of nonabsorbable Embosphere Microspheres (BioSphere Medical, Inc, Rockland, MA, USA) or absorbable agents (Avitene Microfibrillar Collagen Hemostat; MedChem Products, Inc, Woburn, MA, USA; or gelfoam particles 1–2 mm in size; Pharmacia & Upjohn Company, Kalamazoo, MI, USA). All patients received both epirubicin (Pharmacia & Upjohn S.p.A, Milan, Italy) and mitomycin C (Kyowa Hakko Kogyo Co, Ltd, Tokyo, Japan) as chemotherapeutic agents. The doses of chemotherapeutic agents and the amount of lipiodol depended on the size of the tumor. The choice of using nonabsorbable or absorbable embolic agent depended on the sizes and the patterns of the supplying arteries.

**Table 1** Basic characteristics of patients

Items	Chronic hepatitis B ( $n = 17$ )	Chronic hepatitis C ( $n = 27$ )	Total ( $n = 44$ )
Sex (male/female)*	15/2	16/11	31/13
Age (y)*	56, 37–78	70, 51–85	65, 37–85
Child-Pugh class (A/B)	14/3	24/3	38/6
HCC staging			
I	7	7	14
II	4	15	19
IIIA	6	3	9
IIIC		1	1
IV		1	1

Data for continuous variables are expressed as median and range. The Mann-Whitney test, Fisher's exact test, or Chi-square test was applied for statistical analysis. HCC staging is based on the American Joint Committee on Cancer TNM staging system.

\* $p$  value < 0.05.

HCC = hepatocellular carcinoma.

TACE was completed if all detectable supplying arteries of the tumors were embolized followed by the confirmation of immediate post-TACE angiography. The whole procedure was carried out by an independent radiologist who was blind to the study. Rapid recurrence of HCC was defined as an increase of tumor size >1 cm and/or new development of intrahepatic or metastatic nodule. None of the patients received additional TACE within 3 months after TACE.

### Quantitative determination of serum HBV DNA and HCV RNA levels

Serum HBV DNA and HCV RNA levels were measured quantitatively 1 day before and 3 months after TACE. The serum used for the study was collected immediately from the peripheral veins of the patients and was stored at  $-20^{\circ}\text{C}$  for further investigation. HBV DNA levels were measured by the nucleic acid amplification test (COBAS AmpliPrep/COBAS TaqMan HBV Test; Roche, Branchburg, NJ, USA). The dynamic range was between 20 IU/mL and  $1.1 \times 10^8$  IU/mL. The HCV RNA levels were measured by real-time polymerase chain reaction assay (Abbott RealTime HCV; Abbott, Des Plaines, IL, USA). The lowest detection limit was 30 IU/mL. All HBV DNA or HCV RNA measurements were performed at the same time by the same operator using the same viral assay. Twofold or more increases in serum HBV DNA or HCV RNA levels after TACE were considered as significant increases in viral load caused by TACE [16], whereas decreasing of more than half of pre-TACE serum HBV DNA or HCV RNA levels was considered as a significant decrease of the viral loads. Serum HBV DNA and HCV RNA levels were logarithmically transformed ( $\log_{10}$ ) for statistical analysis.

### Determination of epirubicin and mitomycin C concentrations in plasma

Plasma concentrations of epirubicin and mitomycin C were measured at 1 hour and at 72 hours after TACE. Plasma concentrations of epirubicin and mitomycin C were detected by high-performance liquid chromatography (HPLC) system using Hitachi pump L-2130 and Hitachi UV-Vis detector L-2420 (Hitachi High-Technologies Corporation, Tokyo, Japan) with Waters Model 717 Plus HPLC autoinjector (American Instrument Exchange, Inc, Haverhill, MA, USA). The results were analyzed by Hitachi Model D-2000 chromatography Data Station software (Hitachi High-Technologies Corporation, Tokyo, Japan). To prevent high protein concentrations in samples affecting the HPLC separation, liquid-liquid extraction was used for clean-up and preconcentration of the plasma samples. The detection limit for both epirubicin and mitomycin C was 2 ng/mL. The coefficient of variation values for intra-assay (calculation from five measurements) were 3.5% and 7.6% for epirubicin and mitomycin C, respectively.

### Statistical analysis

Data were analyzed using JMP 7.0 software (SAS Institute, Cary, NC, USA). All data for continuous variables were expressed as median and range. The Mann-Whitney test was

used to compare the difference between medians of continuous variables. The Fisher's exact test or Chi-square test was used to compare proportions between groups. Correlation analysis was investigated by the calculation of a correlation coefficient ( $r$ ). A receiver operating characteristic curve was used to establish the cutoff level that provided the maximal diagnostic accuracy. Multiple logistic regressions were used for multivariate analysis. The statistical significance was defined as  $p < 0.05$ .

### Results

There was no significant difference in baseline viral loads between patients with HCC staging I and  $\geq$ II (4.403, 2.712–7.511 vs. 5.318, 1.146–7.532,  $p = 0.601$ , for CHB; 2.901, 1.465–4.419 vs. 2.909, 0.495–3.558,  $p = 0.525$ , for CHC), whereas the baseline HBV DNA viral loads ( $r = 0.528$ ,  $p = 0.029$ ), but not HCV RNA viral loads ( $r = -0.022$ ,  $p = 0.914$ ), were significantly correlated with serum alanine aminotransferase (ALT) levels before TACE. Similarly, only HBV DNA viral loads ( $r = 0.482$ ,  $p = 0.05$ ), but not HCV RNA viral loads ( $r = 0.192$ ,  $p = 0.336$ ), remained to have a significant correlation with serum ALT levels 3 months after TACE.

A total of 29 patients ( $n = 13$  for CHB and  $n = 16$  for CHC) showed significant changes of the viral loads after TACE. Among them, 26 patients (89.7%) had detectable chemotherapeutic agents within 72 hours after TACE. HBV DNA levels were significantly increased in five (29.4%) and decreased in eight (47.1%) patients, whereas HCV RNA levels were significantly increased in eight (29.6%) and decreased in eight (29.6%) patients. Changes of viral loads after TACE did not significantly differ between CHB and CHC patients ( $p > 0.1$ ). CHB patients with increased HBV DNA levels were older ( $p = 0.045$ ), had lower pre-TACE viral loads ( $p = 0.03$ ), and had higher incidence of pre-TACE white blood cell counts lower than the normal limit ( $p = 0.031$ ) (Table 2). Patients with increased HBV or HCV viral loads were older (71 years, 49–85 years vs. 57 years, 37–84 years,  $p = 0.041$ ); had a higher incidence of pre-TACE white blood cell counts lower than the normal limit (decreased/normal counts, 8/5 vs. 3/13,  $p = 0.023$ ); and had higher plasma mitomycin C concentrations (5.1 ng/mL vs. 2.1 ng/mL,  $p = 0.039$ ) (Fig. 1) than the patients with decreased viral loads. There was no significant difference in plasma epirubicin concentrations between patients with increased and decreased viral loads after TACE (5.4 ng/mL, 0–28.6 ng/mL vs. 3.8 ng/mL, 0–65.8 ng/mL,  $p = 0.86$ ) (Fig. 2). Multiple logistic regressions using age, decreased or normal pre-TACE white blood cell counts, mitomycin C concentrations  $>3.95$  ng/mL selected by receiver operating characteristic curve ( $p = 0.037$ ), and epirubicin concentrations have shown that decreased pre-TACE white blood cell counts was the only significant item associated with increased viral loads after TACE ( $p = 0.048$ ) (Table 3). Seven patients showed increased ALT levels to  $>100$  IU/L 3 months after TACE. Among them, only two patients (one CHB and one CHC) had increased viral loads after TACE. There was no significant association between increased viral loads and increased ALT levels to  $>100$  IU/L 3 months after TACE ( $p > 0.05$ ). Fifteen patients experienced rapid

**Table 2** Characteristics of the 29 patients whose viral loads changed after TACE

Items	HBV DNA levels		HCV RNA levels	
	Increased (n = 5)	Decreased (n = 8)	Increased (n = 8)	Decreased (n = 8)
Sex (male/female)	4/1	7/1	5/3	7/1
Age (y)	68*, 49–78	52*, 37–68	72, 51–85	63, 52–84
Child-Pugh class (A/B)	5/0	7/1	8/0	5/3
HCC staging				
I	2	4	2	2
II	1	1	4	4
IIIA	2	3	1	1
IIIC				1
IV			1	
Log <sub>10</sub> baseline viral loads (IU/mL)	3.78**, 2.71–5.1	6.12**, 3.61–7.53	4.72, 3.5–6.01	6.08, 4.21–7.42
Pre-TACE white blood cell count (decreased/normal)	4/1***	1/7***	4/4	2/6
Leaked plasma mitomycin C concentration (ng/mL)	4.5, 0–15.7	1.3, 0–7.2	5.35, 0–9.4	2.1, 0–5.6
Leaked plasma epirubicin concentration (ng/mL)	3.5, 0–18	1.8, 0–11.3	7.2, 0–28.6	7.84, 0–65.8

Data for continuous variables are expressed as median and range. The Mann-Whitney test, Fisher’s exact test, or Chi-square test was applied for statistical analysis. HCC staging is based on the American Joint Committee on Cancer TNM staging system. The higher plasma concentration of mitomycin C or epirubicin in two measurements was applied for analysis.

\**p* value = 0.045; \*\**p* = 0.03; \*\*\**p* = 0.031.

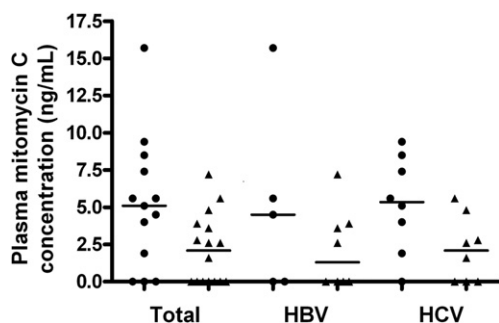
HBV = hepatitis B virus; HCC = hepatocellular carcinoma; HCV = hepatitis C virus; TACE = transcatheter arterial chemoembolization.

recurrence of HCC within 3 months after TACE (*n* = 5 for CHB and *n* = 10 for CHC). There was no significant association between rapid recurrence of HCC and increase of viral loads (*p* > 0.05) (Table 4).

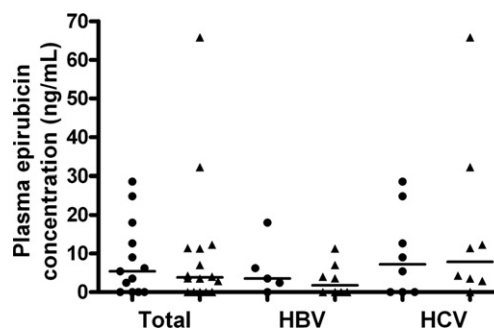
**Discussion**

Whether TACE can affect viral replication remains a source of significant debate. A retrospective study performed by

Jang et al. [14] has reported that TACE could reactivate HBV. However, further studies, including one retrospective [15] and one prospective study [16], suggested that the risk of HBV reactivation after TACE was low. Moreover, Xu et al. [17] have shown that TACE could decrease HBV DNA levels in patients with HCC, especially for those with high pre-TACE HBV DNA levels. The cause of this discrepancy is not clear. These controversial results were also noted in the present study. HBV DNA and HCV RNA viral loads might either increase or decrease after TACE. The present results demonstrated that pre-TACE white blood cell count was the



**Figure 1.** Plasma mitomycin C concentrations after transcatheter arterial chemoembolization. The higher plasma concentration of mitomycin C in two measurements was applied for analysis. ●, increased viral loads after chemoembolization; ▲, decreased viral loads after chemoembolization. *p* = 0.039 for total and *p* > 0.05 for HBV and HCV (Mann-Whitney test). HBV = hepatitis B virus; HCV = hepatitis C virus.



**Figure 2.** Plasma epirubicin concentrations after transcatheter arterial chemoembolization. The higher plasma concentration of epirubicin in two measurements was applied for analysis. ●, increased viral loads after chemoembolization; ▲, decreased viral loads after chemoembolization. *p* > 0.05 for total, HBV, and HCV (Mann-Whitney test). HBV = hepatitis B virus; HCV = hepatitis C virus.

**Table 3** Odds ratios for increased hepatitis B virus or hepatitis C virus viral loads after TACE, calculated by multiple logistic regressions

Items	Odds ratio	<i>p</i> (likelihood ratio test)
Age (y)	1.075484	0.1369
Decreased pre-TACE white blood cell counts vs. normal counts	7.4440162	0.0479
Leaked mitomycin C concentrations >3.95 ng/mL vs. ≤3.95 ng/mL	3.2954142	0.265
Leaked epirubicin concentrations (ng/mL)	0.988408	0.7283

The higher plasma concentration of mitomycin C or epirubicin in two measurements was applied for analysis. TACE = transcatheter arterial chemoembolization.

only significant factor for the reactivation of the replication of HBV or HCV viral loads. This indicates that the status of host defense is the major determinant for viral replication after TACE. Although age and leaked mitomycin C concentrations were not statistically significant determinants for the reactivation of viral replication after TACE determined by multiple logistic regressions, these two factors might still have a potential to affect the viral replication after TACE. Aging is a potential unfavorable factor of immune dysfunction because of impairment of T-cell production or responsiveness [19,20]. Unlike epirubicin, which is less myelotoxic [21], bone marrow suppression is the most common and severe toxic side effect of mitomycin C [22]. Although the leaked plasma mitomycin C concentrations were low in comparison with systemic chemotherapy, the host immune status might also be suppressed by the leaked mitomycin C. No statistical significance of age and leaked mitomycin C as risk factors for the reactivation of viral replication may be because of the small sample size of this study.

Increase of HBV DNA viral loads can initiate host immune response and also cause elevation of ALT levels [23,24]. HBV reactivation after chemotherapy or immunosuppressive therapy might follow the two steps. First, immune suppression results in viral replication. Second, restoration of immune function after withdrawal of immune suppression causes immune-mediated destruction of HBV-infected hepatocytes [25]. Although our results also showed weak correlation between HBV DNA levels and ALT levels, there was no significant association between increased viral loads and increased ALT levels to >100 IU/L 3 months after TACE. This result was in accordance with previous studies [15,16]. No significant reactivation of CHB in patients with increased viral loads after TACE may partially be explained by their

inadequate host responses. This can be supported by the fact that these patients had a higher incidence of pre-TACE white blood cell counts lower than the normal limit. This might also be one of the explanations for no significant reactivation of CHC in patients with increased viral loads after TACE.

HBV and HCV viral loads may fluctuate during the natural course of infection, which was the result from the interaction between host defense and viruses. In the present study, 15 patients did not show significant changes in their viral loads after TACE based on our criteria. However, this did not mean that the viral loads in these patients had absolutely no change. Small variation in viral loads after TACE may be because of either the natural course of CHB or CHC or the influence of TACE. Analysis of their data may cause bias. Therefore, the definition of the change of viral loads in the present study was based on the previous study [16] with stricter criteria that would lessen the bias. Theoretically, the present study would be better to recruit CHB or CHC patients with untreated HCC as a control group. However, it is very difficult to select the same status of host defense in both control and experimental groups. Moreover, it would be unethical to withdraw possible anticancer therapy in CHB or CHC patients with HCC. Therefore, definitive proof of the change of viral loads in HBV/HCV-related HCC is unlikely to obtain from prospective controlled trials.

In conclusion, patients with decreased pre-TACE white blood cell counts have a potential risk of the reactivation of the replication of HBV or HCV after TACE. Whether old age and leaked mitomycin C concentration are associate determinants for the reactivation of HBV and HCV replication after TACE needs further investigation with larger sample size.

**Table 4** Association between rapid recurrence of hepatocellular carcinoma and increase of viral loads after transcatheter arterial chemoembolization

Items	Recurrence (+) ( <i>n</i> = 15)	Recurrence (-) ( <i>n</i> = 29)	Total ( <i>n</i> = 44)
Hepatitis B virus DNA levels			
Increased/no change or decreased ( <i>n</i> )	3/2*	2/10*	5/12
Hepatitis C virus RNA levels			
Increased/no change or decreased ( <i>n</i> )	4/6**	4/13**	8/19

The Fisher's exact test was applied for statistical analysis.

\**p* value = 0.117; \*\**p* = 0.316.

## Acknowledgments

This work was supported by grants from Kaohsiung Medical University Hospital (KMUH96-6G25 and KMUH96-6G26) and the Department of Health, Executive Yuan, ROC (Taiwan) (DOH100-TD-C-111-002). The authors appreciate Yi-Hsin Yang and Jung-San Chang for their help in statistical analysis of the data.

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