hepatocytes, by which means HBV could evade innate immune detection and response.

Supplementary Data

Supplementary data are available at *The Journal of Infectious Diseases* online.

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Impact of Radiation and Hepatitis Virus Infection on Risk of Hepatocellular Carcinoma

Waka Ohishi,[1] Saeko Fujiwara,[1] John B. Cologne,[2] Gen Suzuki,[3] Masazumi Akahoshi,[4] Nobuo Nishi,[5] Masataka Tsuge,[6] and Kazuaki Chayama[6]

In cohort studies of atomic bomb survivors and Mayak nuclear facility workers, radiation-associated increases in liver cancer risk were observed, but hepatitis B virus (HBV) and hepatitis C virus (HCV) infections were not taken strictly into account. We identified 359 hepatocellular carcinoma (HCC) cases between 1970 and 2002 in the cohort of atomic bomb survivors and estimated cumulative incidence of HCC by radiation dose. To investigate contributions of radiation exposure and hepatitis virus infection to HCC risk, we conducted a nested case-control study using sera stored before HCC diagnosis in the longitudinal cohort of atomic bomb survivors. The study included 224 HCC cases and 644 controls that were matched to the cases on gender, age, city, and time and method of serum storage, and countermatched on radiation dose. The cumulative incidence of HCC by follow-up time and age increased significantly with radiation dose. The relative risk (RR) of HCC for radiation at 1 Gy was 1.67 (95% confidence interval: 1.22-2.35) with adjustment for alcohol consumption, body mass index (BMI), and smoking habit, whereas the RRs for HBV or HCV infection alone were 63 (20-241) and 83 (36-231) with such adjustment, respectively. Those estimates changed little when radiation and hepatitis virus infection were fit simultaneously. The RR of non-B, non-C HCC at 1 Gy was 1.90 (1.02-3.92) without adjustment for alcohol consumption, BMI, or smoking habit and 2.74 (1.26-7.04) with such adjustment. ***Conclusion:*** **These results indicate that radiation exposure and HBV and HCV infection are associated independently with increased HCC risk. In particular, radiation exposure was a significant risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, BMI, or smoking habit. (HEPATOLOGY 2011;53:1237-1245)**

Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide, and chronic infections with hepatitis B virus (HBV) or hepatitis C virus (HCV) are recognized as critically important risk factors for HCC. Our previous study actually showed that about 63% of HCC in atomic bomb survivors is related to HCV infection, 14% to HBV infection, and 2% to both HBV and HCV infections.[1] However, an increase of non-B, non-C HCC without HBV and HCV infection has been noted recently in Japan.[2,3] The etiology of non-B, non-C HCC has been poorly understood, although alcoholic hepatitis, nonalcoholic fatty liver disease (NAFLD) including nonalcoholic steatohepatitis (NASH), and hemochromatosis[4,5] are known as risk factors. In Japan, NAFLD has increased along with Westernization of lifestyle, and most NASH cases have developed due to such lifestyle-related diseases such as obesity, diabetes mellitus, and hyperlipidemia.[6] Obesity and diabetes mellitus, as well as NAFLD, have also recently received increased attention as risk factors for HCC.[1,7-12]

Abbreviations: AHS, Adult Health Study; BMI, body mass index; CI, confidence interval; ERR: excess relative risk; HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; NAFLD, nonalcoholic fatty liver disease; NASH, nonalcoholic steatohepatitis; RERF, Radiation Effects Research Foundation; RR, relative risk.

From the [1]Department of Clinical Studies, and [2]Statistics, Radiation Effects Research Foundation, Hiroshima, Japan; [3]International University of Health and Welfare Clinic, Ohtawara City, Tochigi, Japan; [4]Department of Clinical Studies, Radiation Effects Research Foundation, Nagasaki, Japan; [5]Center for Collaboration and Partnership, National Institute of Health and Nutrition, Tokyo, Japan; [6]Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, Hiroshima, Japan.

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Address reprint requests to: Waka Ohishi, M.D., Ph.D., Department of Clinical Studies, Radiation Effects Research Foundation, 5-2 Hijiyama Park, Minami-ku, Hiroshima, 732-0815, Japan. E-mail: nwaka@rerf.or.jp; fax: 81-82-261-3259.

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An increased risk of liver cancer with radiation dose among atomic bomb survivors has been reported based on tumor registries, mortality studies, and pathology review,[13-16] but hepatitis virus infection status was not taken into account. In three previous HBV studies at the Radiation Effects Research Foundation (RERF), the HBV surface antigen (HBsAg)-positive proportion increased with radiation dose.[17-19] Previous research at RERF demonstrated no increase in the prevalence of anti-HCV antibody (anti-HCV Ab) with radiation dose,[20] but reported supermultiplicative effects between radiation exposure and chronic HCV infection in the etiology of HCC without cirrhosis.[21]

On the other hand, the cohort study in workers at the Mayak nuclear facility demonstrated that the risk of liver cancer mortality was significantly associated with plutonium exposure,[22] and that the incidence of HCC was marginally significantly associated with plutonium exposure.[23] In the latest analysis, a significant plutonium dose-response relationship was observed for liver cancer mortality, with risk reasonably described by a linear function.[24] However, liver cancer in those analyses included hepatoblastoma and intrahepatic cholangiocarcinoma as well as HCC. In addition, hepatitis virus infection status was not taken into account in a strict and in-depth manner, although HCC accounted for most of the liver cancer.

A lifespan study using B6C3F1 mice exposed to continuous low-dose-rate γ rays demonstrated that the incidence of HCC was significantly increased in male mice exposed to total doses equivalent to 8,000, 400, and 20 mGy and in females exposed to 8,000 mGy. However, the incidence of other liver tumors did not significantly increase except for that of hepatoblastoma in males exposed to 400 mGy.[25]

With the aim of determining whether radiation exposure is an independent risk factor for HCC, even after adjusting for hepatitis virus infection, alcohol consumption, body mass index (BMI), and smoking habit, we conducted a nested case-control study among atomic bomb survivors using stored sera. We also evaluated whether radiation, alcohol consumption, increase of BMI, and smoking habit contribute to increased risk for non-B, non-C HCC.

Patients and Methods

Cohorts. The Atomic Bomb Casualty Commission (ABCC) and its successor, the RERF, established the Adult Health Study (AHS) longitudinal cohort in 1958, in which more than 20,000 gender-, age-, and city-matched proximal and distal atomic bomb survivors and persons not present in the cities at the time of bombings are examined biennially in outpatient clinics in Hiroshima and Nagasaki.

Cases and Controls. Incident cancer cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, supplemented by additional cases detected by way of pathological review of related diseases.[26] As described in our previous study,[1] 359 primary HCC cases were diagnosed among 18,660 AHS participants between 1970 and 2002 who visited our outpatient clinics before their diagnosis. Of these, 229 cases had serum samples obtained within 6 years before HCC diagnosis. After excluding five cases with inadequate stored serum, 224 cases remained for our study. There were no important differences in characteristics such as gender, age at HCC diagnosis, city, alcohol consumption, BMI, or radiation dose to the liver (among exposed persons) between HCC cases excluded due to nonavailability of stored serum and those included in the present study.

Three control sera per case were selected from the at-risk cohort members matched on gender, age, city, and time and method of serum storage, and countermatched on radiation dose in nested case-control fashion.[27] Countermatching (to increase statistical efficiency for studying joint effects of radiation and other factors) was performed using four strata based on whole-body (skin) dose: zero dose (<0.0005 Gy), <0.05 Gy, <0.75 Gy, and ≥ 0.75 Gy (nonzero categories correspond roughly to tertiles of skin dose among all eligible exposed cases). At the time of each case diagnosis, one control serum was selected for each of the three dose strata not occupied by the case. Although the total number of potential matched control serum samples is 672, due to occasional lack of subjects with stored sera who met the matching and countermatching criteria, the total number of control serum samples actually selected was 644, which comprised 488 sera from unique noncase subjects and 156 sera from subjects sampled on repeated occasions.

Laboratory Tests. Virological assays were performed on 211 case and 640 control sera, because 13 case samples and four control samples had insufficient stored sera for these assays. HBsAg and antibody to hepatitis B core antigen (anti-HBc Ab) were measured by enzyme immunoassay (EIA), and anti-HCV Ab was measured by second-generation EIA as described.[28,29] Qualitative detection of HCV RNA among anti-HCV-positive samples was performed using a thermocycler (Whatman Biometra, Goettingen, Germany) based on the nested polymerase chain reaction (PCR) method, as described.[29] HBV infection (HBV+) status was

defined as positive for HBsAg or having a high titer of anti-HBc Ab. HCV infection (HCV+) status was defined as positive for HCV RNA. Non-B, non-C status was defined as negative for HBsAg and not having a high titer of anti-HBc Ab (HBV−) as well as negative for HCV RNA (HCV−).

Radiation Dose. Radiation dose to the liver was estimated for each subject according to Dosimetry System DS02.[30] A weighted sum of the gamma dose in gray plus 10 times the neutron dose in gray was used. Because of the countermatched selection of cases, direct comparison of doses between cases and controls in the study requires that control doses be weighted by the inverses of their selection probabilities.

Information on Alcohol Consumption, BMI, and Smoking Habit. Information on alcohol consumption was obtained from the 1965 AHS questionnaire when available, with missing data complemented using the 1978 mail survey. Alcohol consumption was quantified as volume of each type of alcoholic beverage; mean ethanol amounts were calculated as grams per day as described.[31] BMI (kg/m^2) was calculated from height and weight measured at the AHS examination. Subjects were classified based on BMI quintiles with cutpoints of 19.5, 21.2, 22.9, and 25.0. Following the recommendations for Asian people by the World Health Organization (WHO), the International Association for the Study of Obesity, and the International obesity Task Force,[32] 21.3 to 22.9 kg/m^2 was considered normal, 23.0 to 25.0 kg/m^2 as overweight, and >25.0 kg/m^2 as obese. We used information on BMI obtained 10 years before the time of HCC diagnosis or control matching because this condition is subject to change due to disease progression in the later stages before development of HCC. Information on smoking habit was obtained from the 1965 questionnaire; subjects were categorized as never, current (at time of survey), or former smoker.

Ethical Considerations. This study (RERF Research Protocol 1-04) was reviewed and approved by the Research Protocol Review Committee and the Human Investigation Committee of RERF.

Statistical Analyses. The nested case-control design was analyzed using a partial likelihood method analogous to that used for cohort follow-up studies,[33] which is in practice the same as the conditional binary data likelihood for matched case-control studies[34] except that the subjects (cases and "controls") in the study are not completely independent due to repeated selection. Cumulative incidence of HCC by follow-up time (year) and age was derived according to the method of Nelson and Aalen, using Cox regression to adjust for age at start of follow-up. Cumulative incidence by radiation dose groups (0-0.0009, 0.001-0.999, and 1.0+ Gray) was compared using the Gehan/Breslow generalized Wilcoxon test. All factors other than radiation were analyzed using relative risks (RRs) estimated by a log-linear model. Although radiation exposure could have been adjusted by matching on radiation dose as an additional matching factor in the control selection,[35] in addition to assessing effects of lifestyle factors and viral hepatitis, another purpose of the present study was to examine the effects of radiation exposure after adjustment for possible confounding and interaction by these factors, so matching on radiation—which precludes analysis of radiation risk—was not desirable; rather, we countermatched on radiation.[27,33,36] Radiation risk was analyzed using an excess relative risk (ERR) model (ERR = RR-1) as done previously.[37] The cumulative hazard estimator and comparisons by radiation dose groups were computed using Stata (StataCorp, College Station, TX; v. 11.1); all other analyses were conducted using Epicure (HiroSoft International, Seattle, WA; v. 1.81).

Results

Characteristics of Cases and Controls. Characteristics of the 224 HCC cases and 644 matched controls are shown in Table 1. HCC cases and controls were comparable with respect to gender, age, city, and time and method of serum storage by design. Prevalence of HBV and/or HCV infection status in HCC cases is higher than those in controls. Higher proportions of HCC cases had a history of alcohol consumption of more than 40 g of ethanol per day, were obese (BMI >25.0 kg/m^2), and were current smokers, compared with the controls. HCC cases also received on average higher radiation doses to the liver, compared with the controls.

Cumulative Incidence of HCC by Radiation Dose. Figure 1A,B shows the cumulative incidence of HCC by radiation dose using either follow-up time (adjusted for age at start of follow-up) or age. Of 359 HCC cases diagnosed among 18,660 AHS subjects between 1970 and 2002, the analysis was performed using 322 HCC cases, based on 16,766 subjects with known radiation dose. A significant increase with radiation dose was seen with cumulative incidence both by follow-up time ($P = 0.028$) (Fig. 1A) and by age ($P = 0.0003$) (Fig. 1B). The effect of radiation was especially evident at age 60 years or later.

Risk of HCC for Radiation and Hepatitis Virus Infection. Table 2 shows risk of HCC with and without adjustment for categorical alcohol consumption,

Table 1. Characteristics of HCC Cases and Controls

Study Variables	HCC Cases		Controls	
	Number with Complete Data	n (%)	Number with Complete Data	n (%)
Matched variables				
Gender	224		644	
Male		136 (60.7)		387 (60.1)
Female		88 (39.3)		257 (39.9)
Age at HCC diagnosis (yr)	224	67.6 (10.1)*	–	–
City	224		644	
Hiroshima		155 (69,2)		444 (68.9)
Nagasaki		69 (30.8)		200 (31.1)
Age at serum storage (yr)	224	66.4 (10.2)*	644	63.7 (9.8)*
Unmatched variables				
Viral etiology	211		640	
HBV−/HCV −		45 (21.3)		579 (90.5)
HBV+/HCV −		29 (13.7)		18 (2.8)
HBV−/HCV +		132 (62.6)		41 (6.4)
HBV +/HCV +		5 (2.4)		2 (0.3)
Alcohol consumption (g ethanol/day)	199		577	
None		97 (48.7)		315 (54.6)
0< <20		37 (18.6)		130 (22.5)
20≤ <40		20 (10.1)		64 (11.1)
≥40		45 (22.6)		68 (11.8)
BMI (kg/m^2)	210		633	
10 yrs before diagnosis				
≤19.5		38 (18.1)		122 (19.3)
19.6 - 21.2		33 (15.7)		136 (21.5)
21.3 - 22.9		36 (17.2)		142 (22.4)
23.0 - 25.0		49 (23.3)		124 (19.6)
>25.0		54 (25.7)		109 (17.2)
Smoking habit	199		578	
Never		80 (40.2)		283 (49.0)
Current smoker		107 (53.8)		262 (45.3)
Former smoker		12 (6.0)		33 (5.7)
Radiation dose to the liver (Gy)	204	0.46 (0.69)*	606	0.34 (0.56)*,†

*Mean (SD).
†Weighted mean radiation dose (among controls), calculated by weighting according to their countermatching selection probabilities.

BMI, and smoking habit based on all cases of HCC. The analysis was performed using 186 HCC cases and 600 controls, both separately (radiation only or hepatitis virus infection only) and jointly (radiation and hepatitis virus infection were fit simultaneously), based on subjects with known radiation dose and known HBV and HCV infection status. In analyses where effects of radiation and hepatitis virus infection were fitted separately, unadjusted RR at 1 Gy of HCC for radiation was 1.40 (95% confidence interval [CI], 1.07-1.89, $P = 0.013$), whereas unadjusted RRs of HCC for HBV+/HCV− status and HBV−/HCV+ status were 34 (95% CI, 13-106, $P < 0.001$) and 57 (95% CI, 27-140, $P < 0.001$), respectively. After adjustment for categorical alcohol consumption, BMI, and smoking habit, significant association was found between HCC and radiation dose or hepatitis virus infection, resulting in an RR at 1 Gy of 1.67 (95% CI, 1.22-2.35, $P < 0.001$) for radiation and RRs of 63 (95% CI, 20-241, $P < 0.001$) for HBV+/HCV− status and 83 (95% CI, 36-231, $P < 0.001$) for HBV−/HCV+ status. The above estimates changed little when radiation and hepatitis virus infection were fit simultaneously.

Risk of HCC for Radiation After Excluding Persons with Either or Both Hepatitis Virus Infections. After excluding subjects with either or both hepatitis virus infections, the RRs at 1 Gy of HCC for radiation were estimated as shown in Table 3. There were 161 cases including 119 HCV-infected individuals and 452 matched controls including 29 HCV-infected individuals without HBV infection only. There were 66 cases including 24 HBV-infected individuals and 176 matched controls including 5 HBV-infected individuals without HCV infection only. The adjusted analyses indicated that radiation exposure was significantly associated with increased risks for HCC,

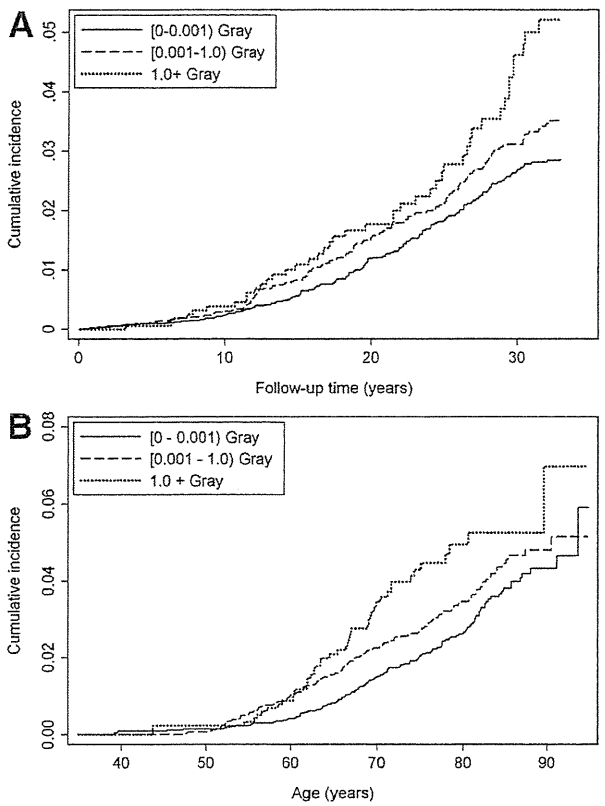

Fig. 1. Cumulative incidence of HCC (1970-2002) by radiation dose. Dotted line: radiation dose ≥1.0 Gy; dashed line: radiation dose 0.001 ≤ <1.0 Gy; solid line, radiation dose 0 ≤ <0.001 Gy. Cumulative HCC incidence by follow-up time (A) and age (B) increased significantly ($P = 0.028$, $P = 0.0003$, respectively) with radiation dose.

even after excluding HBV- or HCV-infected individuals. Furthermore, significant association was found between non-B, non-C HCC and radiation dose, resulting in an RR at 1 Gy of 1.90 (95% CI, 1.02-3.92, $P = 0.041$) for radiation without adjustment for categorical alcohol consumption, BMI, and smoking habit and 2.74 (95% CI, 1.26-7.04, $P = 0.007$) with such adjustment.

Risk of Non-B, Non-C HCC. Effects of alcohol consumption, BMI, and smoking habit on non-B, non-C HCC risk with or without adjustment for radiation dose were estimated using continuous and categorical covariates as shown in Table 4. RRs for continuous covariates are for a one-unit difference in the factor. Risk of non-B, non-C HCC for alcohol consumption per 20 g of ethanol per day was significant with a log-linear model (adjusted RR 1.64, 95% CI, 1.05-2.81, $P = 0.029$), but was limited to the category ≥40 g of ethanol per day (adjusted RR 5.49, 95% CI, 0.98-39.2, $P = 0.052$). Significant log-linear association was not found with continuous BMI, and even the category BMI >25.0 kg/m^2 (obese) 10 years before diagnosis did not evidence significant risk despite a rather large estimate of RR (adjusted RR 3.17, 95% CI, 0.92-12.3, $P = 0.068$). Current smoking evidenced significant risk (adjusted RR 5.95, 95% CI, 1.34-33.2, $P = 0.018$), but there were no continuous data on amount smoked. These results indicate that alcohol consumption per 20 g of ethanol per day, current smoking, and perhaps BMI of >25.0 kg/m^2 10 years before diagnosis are associated independently with increased risk for non-B, non-C HCC.

Discussion

The present study confirmed that radiation is associated with increased incidence of HCC among atomic bomb survivors. Additionally, the nested case-control study indicates that radiation and HBV and HCV infection are associated with increased risk for HCC, and that radiation remains an independent risk factor for HCC after taking into account hepatitis virus infection, alcohol consumption, BMI 10 years before HCC diagnosis, and smoking habit. Furthermore, significant association was observed between non-B, non-C HCC and radiation dose, alcohol consumption, and smoking, whereas obesity 10 years before diagnosis was marginally significantly associated with increased risk for non-B, non-C HCC.

In the analysis (Table 2) in which radiation dose and hepatitis virus infection were fitted separately, radiation was significantly associated with increased risk

Table 2. Risk of HCC for Radiation and HBV or HCV Infection Status

Variables	Number of Cases/Controls	Unadjusted RR (95% CI)		Adjusted* RR (95%CI)	
		Alone†	Joint‡	Alone†	Joint‡
Radiation (at 1Gy)	186/600	1.40 (1.07-1.89)	1.39 (0.93-2.26)	1.67 (1.22-2.35)	1.82 (1.09-3.34)
HBV+/HCV −	24/14	34 (13-106)	30 (11-91)	63 (20-241)	50 (16-184)
HBV−/HCV +	119/35	57 (27-140)	58 (28-147)	83 (36-231)	87 (37-251)

Abbreviations: CI, confidence interval; RR, relative risk.

*Adjusted for categorical alcohol consumption, BMI 10 yrs before diagnosis, and smoking habit.

†Radiation dose to the liver and hepatitis virus infection status were fit separately.

‡Radiation dose to the liver and hepatitis virus infection status were fit simultaneously.

Table 3. Risk of HCC for Radiation After Excluding Persons Infected with HBV and/or HCV

Subjects	Number of Cases/Controls	Unadjusted RR at 1 Gy (95% CI)	Adjusted* RR at 1 Gy (95% CI)
Exclude HBV+ (no HCV adjustment)	161/452	1.48 (1.10-2.05)	1.91 (1.34-2.81)
(adjust for HCV)		1.60 (0.997-2.78)	2.32 (1.25-4.76)
Exclude HCV+ (no HBV adjustment)	66/176	1.61 (1.003-2.76)	1.91 (1.13-3.48)
(adjust for HBV)		1.68 (0.96-3.23)	2.16 (1.12-4.76)
Exclude both HBV+ and HCV+†	42/108	1.90 (1.02-3.92)	2.74 (1.26-7.04)

Abbreviations: CI, confidence interval; RR, relative risk.
*Adjusted for categorical alcohol consumption, BMI 10 yrs before diagnosis, and smoking habit.
†Non-B, non-C status.

for HCC with or without adjustment for alcohol consumption, BMI, and smoking habit. Although this finding is in agreement with our previous understanding that liver cancer risk is significantly associated with radiation without adjustment for hepatitis virus infection among atomic bomb survivors, it is difficult to compare the HCC risk estimates between the previous and current study results.[13-16] The difficulty is caused by the inclusion of hepatoblastoma and intrahepatic cholangiocarcinoma in addition to HCC as liver cancer cases in analyses of tumor registry-based liver cancer risk (ERR at 1 Sv = 0.49),[13] mortality study- and tumor registry-based[15,16] liver cancer mortality risk (male: ERR per Sv = 0.39, female: ERR per Sv = 0.35), and liver cancer risk (male: ERR per Gy = 0.32, female: ERR per Gy = 0.28), despite the fact that the majority of liver cancer cases were HCC. Because a relatively large fraction of liver cancer cases was included that were diagnosed only on the basis of death certificates,[13,16] complete exclusion of metastatic liver tumor cases from such cases may not have been possible. Metastatic liver tumor cases were excluded in an analysis of pathological review-based liver cancer risk (ERR per Gy = 0.81), but hepatoblastoma and intrahepatic cholangiocarcinoma were included with HCC.[14]

In the current analyses adjusted for alcohol consumption, BMI, and smoking habit, the RR estimates for radiation increased slightly and showed statistical significance with adjustment for HBV and HCV infection status. HBV infection may be considered an intermediate risk factor for HCC, because three of four previous HBV screenings demonstrated that HBsAg prevalence increases with radiation dose[17-19,38]; therefore, adjustment for HBV infection status might be expected to result in a decreased radiation risk estimate. However, such interpretation is difficult because

Table 4. Risk of Non-B, Non-C HCC for Alcohol Consumption, BMI, and Smoking Habit

Variables	Number of Cases/Controls	Unadjusted RR (95% CI)†	Adjusted* RR (95% CI)†
Continuous			
Alcohol consumption (per 20 g ethanol per day)	37/96	1.51 (0.98-2.60)	1.64 (1.05-2.81)
BMI 10 yrs before diagnosis (per +1 kg/m^2 difference)	41/107	1.06 (0.95-1.18)	1.06 (0.95-1.19)
Categorical			
Alcohol consumption (g ethanol per day)			
None	22/58	1	1
0 < < 20	5/21	0.98 (0.24-3.60)	0.85 (0.18-3.48)
20 ≤ < 40	2/10	0.78 (0.09-4.49)	0.68 (0.08-4.07)
≥ 40	8/7	5.25 (1.04-33.5)	5.49 (0.98-39.2)
BMI (kg/m^2) 10 yrs before diagnosis			
≤ 19.5	8/18	1.64 (0.45-6.20)	1.66 (0.42-6.83)
19.6 - 21.2	3/22	0.74 (0.12-3.66)	0.80 (0.13-4.15)
21.3 - 22.9	6/25	1	1
23.0 - 25.0	10/24	1.76 (0.42-7.93)	2.37 (0.52-11.5)
> 25.0	14 /18	2.85 (0.86-10.5)	3.17 (0.92-12.3)
Smoking habit			
Never	17/58	1	1
Current smoker	19/38	3.78 (0.99-17.1)	5.95 (1.34-33.2)
Former smoker	1/3	2.83 (0.10-52.3)	4.67 (0.16-93.7)

Abbreviations: CI, confidence interval; RR, relative risk.
*Adjusted for radiation dose to the liver.
†Alcohol consumption, BMI, and smoking habit were fit simultaneously, either as continuous (alcohol and BMI only) or categorical factors.

the risk estimate was also adjusted for HCV infection status, although anti-HCV Ab prevalence is not significantly associated with radiation dose.[20] We therefore examined HBV and HCV infection status and concomitant radiation effects separately, excluding persons with one or the other viral infection.

RRs of HCC for radiation after excluding persons infected with HBV or HCV were generally higher than with the full data, but differed little depending on which virus was used for exclusion (Table 3). As with the full data, adjustment for HBV or HCV infection status reduced the statistical significance of the radiation effect but had little impact on the RR estimates themselves. The RR of HCC for radiation after excluding persons infected with HBV and HCV (i.e., the RR of non-B, non-C HCC for radiation) was significant with or without adjustment for alcohol consumption, BMI, and smoking habit. As there can be no viral mediation of the radiation risk in noninfected individuals, lower radiation risks estimated in infected individuals might be considered evidence of mediation, but mediation would imply that risk decreases with adjustment for viral infection status, which did not occur. The reduction in statistical significance with adjustment for HBV and HCV infection status might be due to loss of power when further parameters for the risks of HCC for hepatitis virus infection are estimated or the number of subjects is reduced by exclusion.

As with the results reported previously,[1] there is evidence that alcohol consumption of $\geq$40 g/day ethanol and BMI >25.0 kg/m^2 10 years before diagnosis are associated with non-B, non-C HCC risk (Table 4). However, the evidence is not as strong given the small amount of data after excluding persons infected with HBV and HCV. The current study demonstrates that smoking is significantly associated with non-B, non-C HCC risk, although lack of continuous data precluded estimation of the relationship to amount smoked. This finding is consistent with recent assessments by the International Agency for Research on Cancer (IARC) where HCC has been positioned as a smoking-related malignant disease.[39] Some studies have shown effects of smoking on risk of HCC, but few studies have incorporated, in a strict and in-depth manner, HBV and HCV infections.[11,40]

Cohort studies of atomic bomb survivors[13-16] and Mayak nuclear facility workers[22-24] have indicated beyond a doubt that radiation increases liver cancer risk, even though hepatitis virus infection was not taken into account. It is also well known that persistent long-term internal exposure to α particles from Thorotrast, a radioactive contrast agent, can induce hemangiosarcoma, cholangiocarcinoma, and HCC in humans.[41-43] Because a significant radiation effect is observed in a high proportion of HCC cases having a p53 mutation, it has been suggested that p53 is one of the intracellular targets of atomic bomb radiation and thus a cause of the increased HCC incidence among atomic bomb survivors.[44] A lifespan study in mice exposed to continuous low-dose-rate γ rays demonstrated that the incidence of HCC was significantly increased, especially in male mice.[25] Liver weights of irradiated mice were significantly greater than those of nonirradiated controls, and the lipid content was significantly increased in irradiated mouse livers.[45] It is considered that hepatic steatosis itself is a state conferring risk for high carcinogenicity, and that in steatohepatitis, oxidative stress due to fatty acid oxidation in hepatocytes may cause DNA injury and eventually lead to carcinogenesis.[46] There is a significant association of radiation dose with prevalence of fatty liver among Nagasaki AHS participants, although a significant association has not been found between obesity (BMI $\geq$26.0 kg/m^2) and radiation dose.[47] These findings may explain part of the mechanism of increased risks of HCC with radiation exposure.

The main strengths of our study include its prospective cohort-based, nested case-control design, which minimizes selection bias, the use of stored sera, and a wealth of epidemiological information obtained prior to HCC diagnosis. It is difficult and expensive to perform full cohort serum analyses, whereas the nested case-control design utilized here can provide substantial reductions in cost and effort with little loss of statistical efficiency.[36] Another major strength of our study is that it incorporated, in a strict and in-depth manner, hepatitis virus infection status and HCC cases were identified through the Hiroshima Tumor and Tissue Registry and Nagasaki Cancer Registry, supplemented by additional cases detected by way of pathological review of related diseases.[26]

A limitation of our study is that the joint effects of radiation and hepatitis virus infection could not be estimated from the standpoint of causality. As discussed previously, HBV and possibly HCV infection may act as intermediate risk factors in radiation-associated HCC. Previous studies have consistently demonstrated that prevalence of HBsAg increases with radiation dose within the AHS,[17-19] although no dose response for anti-HCV Ab has been detected.[20] Therefore, when the risk of HCC for radiation is estimated while controlling for HBV infection, some of the radiation risk may be absorbed in the coefficient for HBV infection. In other words, the radiation risk coefficient

does not represent the radiation effect independent of mediation by HBV infection and the HCC risk for HBV infection itself is not correctly estimated, because the actual causal pathway is not explicitly modeled. In addition, we cannot easily disentangle the joint effects of radiation and HBV infection using standard regression models, because HBV infection is not a true confounding risk factor but an intermediate risk factor. Nevertheless, that the radiation risk did not decrease with concomitant adjustment for viral infection suggests that the practical extent of mediation may be small. We are currently developing methods of statistical analysis that jointly consider the dose response for the intermediate viral factor as well as the joint risk of HCC for both hepatitis virus infection and radiation in the countermatched, nested case-control design.

In conclusion, radiation exposure was associated with increased risk of HCC, even after adjusting for HBV or HCV infection, alcohol consumption, BMI, and smoking habit. Moreover, radiation exposure was an independent risk factor for non-B, non-C HCC with no apparent confounding by alcohol consumption, BMI, or smoking habit. The mechanistic form of joint effects of radiation and HBV or HCV infection on HCC risk could not be estimated, but the development of new statistical methods that jointly consider the dose response for the intermediate viral factor will make such an analysis possible in the future. In particular, in-depth understanding of the mechanisms by which radiation exposure as well as obesity, alcohol drinking, and smoking contribute to development of non-B, non-C HCC may lead to prevention, early detection, and better therapeutic strategies.

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MECHANISMS OF GASTROINTESTINAL, PANCREATIC AND LIVER DISEASES

Animal model for study of human hepatitis viruses

Kazuaki Chayama,*,† C Nelson Hayes,*,† Nobuhiko Hiraga,*,† Hiromi Abe,*,† Masataka Tsuge*,† and Michio Imamura*,†

*Department of Medicine and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate School of Biomedical Sciences, Hiroshima University, and †Liver Research Project Center, Hiroshima, Japan

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Correspondence
Professor Kazuaki Chayama, Department of Medical and Molecular Science, Division of Frontier Medical Science, Programs for Biomedical Research, Graduate school of Biomedical Science, Hiroshima University, 1-2-3 Kasumi, Minami-ku, Hiroshima 734-8551, Japan. Email: chayama@hiroshima-u.ac.jp

Abstract

Human hepatitis B virus (HBV) and hepatitis C virus (HCV) infect only chimpanzees and humans. Analysis of both viruses has long been hampered by the absence of a small animal model. The recent development of human hepatocyte chimeric mice has enabled us to carry out studies on viral replication and cellular changes induced by replication of human hepatitis viruses. Various therapeutic agents have also been tested using this model. In the present review, we summarize published studies using chimeric mice and discuss the merits and shortcomings of this model.

Introduction

Hepatitis B virus (HBV) and hepatitis C virus (HCV) are pathogens that cause chronic infection in humans. There are 360 million and 170 million people infected worldwide with HBV or HCV, respectively.[1,2] Infected individuals develop acute hepatitis, chronic hepatitis and liver cirrhosis. The viruses are also important causative agents of hepatocellular carcinoma, especially in the Asia–Pacific region.[3] Study of the biology and development of therapies for each virus has long been hampered by the lack of a small animal model that supports hepatitis virus infection. This is probably as a result of the lack of receptor molecules necessary for viral infection in animal liver cells.

Transgenic mice that express over-length HBV-DNA export viral particles into the serum,[4] and such animals can be used to evaluate antiviral agents,[5–7] as well as HBV-targeted siRNA[8]. However, the virus life cycle is not established in this model, and it is inappropriate for studying drug-resistant HBV strains. Accordingly, researchers attempted to transplant human hepatocytes into mice. The development of the trimera mouse was one such attempt, in which human hepatocytes were transplanted under the kidney capsule of immune-deficient mice after lethal irradiation.[9,10] However, the number of hepatocytes that could survive on the kidney capsule was small, and normal liver architecture was not present. Although 85% of HBV-inoculated animals developed HBV viremia, the titer was less than 10^5 virus particles or IU/mL.[9] Similarly, 85% of HCV-inoculated animals also developed viremia,[10] but the level of the viremia only reached 10^5/mL. Thus, the advent of human hepatocyte transplanted uPA/scid mice has provided the first really useful model for acute and chronic infections of human hepatitis virus.

Human liver cell transplanted uPA/scid mice

Transgenic mice in which the urokinase gene is driven by the human albumin promoter/enhancer were developed and shown to have accelerated hepatocyte death and consequent chronic stimulation of hepatocyte growth.[11] Transplanted rat hepatocytes proliferated and repopulated injured livers in immunodeficient uPA mice, which were produced by mating uPA transgenic mice with scid mice.[12] Human hepatocytes were then transplanted into uPA/scid mice; these cells proliferated and replaced the apoptotic mice liver cells (Fig. 1).

Such human hepatocyte chimeric mice have been shown to be susceptible to both HBV[16] and HCV[17] infections. Repopulation levels by human hepatocytes have been estimated by measuring human albumin levels in mouse serum. Replication levels of both HBV[13] and HCV[17] were higher in mice in which the repopulation index was higher. A unique attempt to remove mouse residual liver cells with the herpes simplex virus type-1 thymidine kinase (HSVtk)/ganciclovir (GCV) system failed to result in a higher repopulation rate as a result of damage to the transplanted human hepatocyte caused by bystander effects.[18] Despite this, mice with livers that have been highly repopulated with human hepatocytes

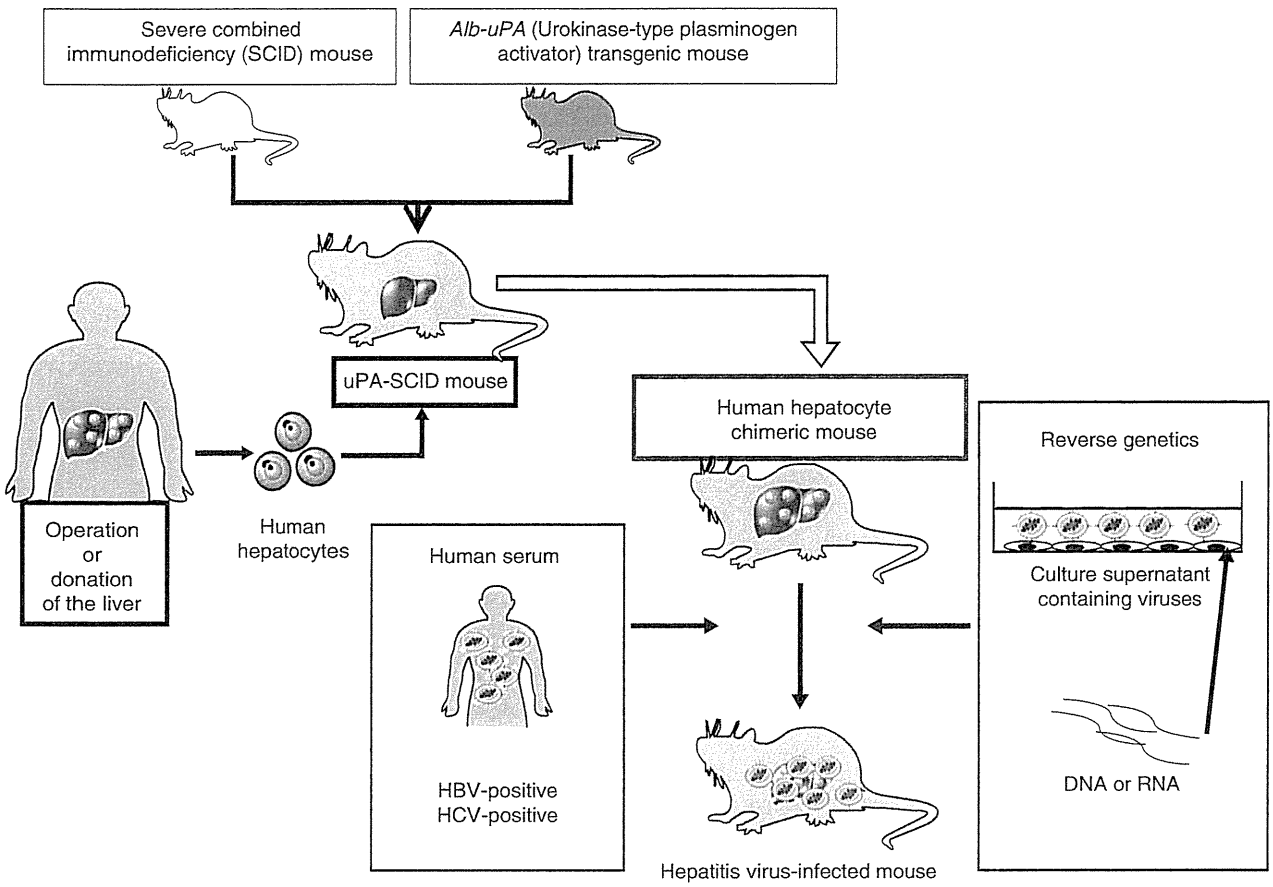

Figure 1 Generation of human hepatocyte chimeric mice and hepatitis virus infection model. A uPA/scid mouse was created by mating uPA transgenic mouse and scid mouse. Human hepatocytes obtained by surgical resection or donation were transplanted to newborn mice. The chimeric mice can be infected with hepatitis B virus (HBV) or hepatitis C (HBC) virus by injecting human serum containing these viruses. Alternatively, the mice can be infected by HBV[13] or HCV[14] created in cell culture or by injecting HCV RNA into the mouse liver.[15]

are susceptible to infection with both HBV and HCV, and as such comprised the most effective small animal model for chronic hepatitis so far developed.[19,20] An example of a highly repopulated mouse liver that we are using in experiments is shown in Figure 2.

Highly repopulated mice have been shown to be a valuable model for the study of drug metabolism.[21–29] Advances in technology for human hepatocyte transplantation have enabled serial passage of human hepatocytes in uPA/scid mice and have been shown to retain infectivity for HBV.[30]

This mouse model and other animal models for the study of hepatitis viruses have been summarized in reviews by Meuleman and Leroux-Roels,[31] Dandri *et al.*,[32,33] Barth *et al.*,[34] and Kneteman and Toso.[35] The present review will focus on key issues and updated information.

Study of hepatitis B virus infection using human hepatocyte chimeric mice

Since the initial reports of successful transmission of HBV to human hepatocyte chimeric mice in 2001 and 2004,[16,27] several researchers have reported transmission of HBV into similar mice.[13,36,37] In these studies, passage experiments studies show that HBV replicating in mice retain infectivity.[13,36] Further, the presence of viral proteins has been shown immunohistochemically in human hepatocytes transplanted into mouse livers, but these are not present in mouse hepatocytes.[13,36,37] Formation of viral particles in infected mouse livers can be shown by electron microscopy.[36,37] Genetically engineered viruses lacking HBe-antigen have also been shown to infect chimeric mice, proving that e antigen is dispensable for viral infection and replication.[13] In contrast, HBx protein has been shown to be indispensable for viral replication.[38] Transcomplementation of HBx protein with hydrodynamic injection restored HBV infectivity in mice. Interestingly, all revertant viruses show a restored ability to express HBx.[38]

By infecting chimeric mice with genotype A, B and C, differing proliferative capacity has been shown between HBV genotypes.[37] In mice infected for a relatively short time, there are no morphological changes in HBV infected mice livers in studies.[13,36] In contrast, the occurrence of liver cell damage has been reported after long-term infection of chimeric mice with HBV[39] or with specific strains of HBV;[40] these findings are consistent with direct cytopathic effects of HBV under certain conditions.

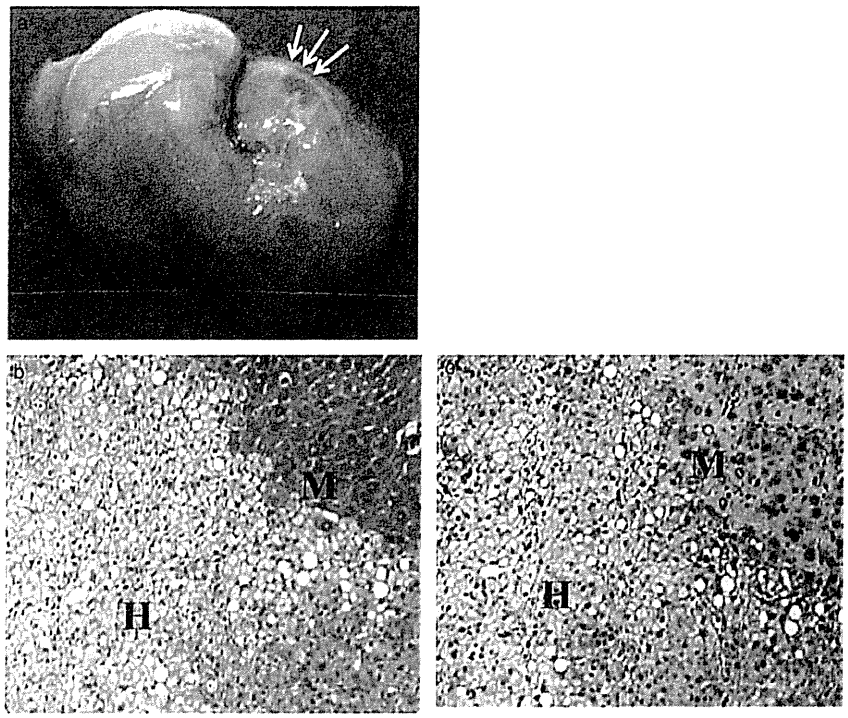

Figure 2 Representative uPA/scid mouse livers repopulated by human hepatocytes. (a) Mouse liver almost completely repopulated by human hepatocytes. Only a small portion of mouse hepatocytes are shown by arrows. (b) Microscopic figure of the mouse liver. M and H indicate regions consisting of mouse and human hepatocytes, respectively (Hematoxylin–eosin staining, magnification: ×100). (c) Microscopic figure of the mouse liver stained with antibody directed against human serum albumin.

The biological properties of a newly identified unique strain of HBV, genotype G, which replicates only in the presence of another genotype, were confirmed using the chimeric mouse.[41] Infectivity of another novel HBV strain, identified from a Japanese patient, that is divergent from known human and ape HBV has also been confirmed.[42] Titration of HBV infectivity, which previously could only be carried out using chimpanzees, can be carried out effectively using chimeric mice.[43]

Taking advantage of the absence of human immune cells in the chimeric mice, Noguchi *et al.*[44] showed that hypermutation of HBV increases in human hepatocytes under interferon treatment. Dandri *et al.* measured viral half-life in human and chimeric mice repopulated with wooly monkey hepatocytes.[45] The results clearly showed that viral half-life is shortened by immunological mechanisms in humans with low viral levels, but not in chimeric mice where functional immunity is absent. Hiraga *et al.*[46] showed an absence of interference between HBV and HCV.

Evaluation of therapeutic agents is the most important role for this mouse model. Tsuge *et al.*[13] assessed the effect of interferon and lamivudine using chimeric mice. Similarly, Dandri *et al.*[47] showed the effects of adefovir using uPA/scid mice repopulated with tupaia hepatocytes, which also support replication of human HBV. Oga *et al.*[48] identified a novel lamivudine-resistant variant that has an amino acid substitution outside of the YMDD motif. They showed that lamivudine was ineffective against the novel mutant strain. It is thus apparent that this mouse/human liver chimeric model is ideal to study the susceptibility of mutant strains to various drugs, because mutant viruses can easily be made and infected into chimeric mice.[13] The model has also been utilized to evaluate viral entry inhibitors derived from the large envelope protein.[49]

Study of hepatitis C virus using human hepatocyte chimeric mice

As observed in studies on HBV, HCV infection efficiency was poor and levels of viremia were low in mice where the repopulation rate of the mouse liver with human hepatocyte was low.[17,50] As shown in Figure 3, human albumin levels in mouse serum were significantly higher in mice in which measurable viremia developed (Hiraga *et al.* unpublished data). Recent studies have therefore been carried out using highly repopulated mice. The usefulness of a newly developed HCV assay,[51] and infectivity of a newly identified intergenotypic recombinant strain,[52] have been reported using the chimeric mice.

Using the remarkable replication ability of the JFH1 genotype 2a strain,[53] infectivity of JFH1 or intergenotypic chimeric viral particles, previously shown in cell culture, has now been shown to be infectious in chimeric mice.[54–56] Infectivity of viruses that were replicated in chimeric mice in cell culture has also been shown, and virus fitness has been studied.[55,56] The role of the HCV core+1 open reading frame and core *cis*-acting RNA elements has also been examined using the chimeric virus.[57] These elegant studies have the limitation that the non-structural part of the virus is limited to that of JFH1. Hiraga *et al.*[14] have shown that infectious clones of genotype 1a and JFH1 can be infected with direct injection of *in vitro* transcribed RNA into the mouse liver.[14] Similarly, Kimura *et al.*[15] reported the establishment of infectious clones of genotype 1b and ablation of RNA polymerase by site-directed mutagenesis abolish infectivity. These infectious clones will be useful for the study of drug-resistant strains.

The model of HCV infection has also been used to show that infection of the virus can be prevented by antibodies against

Table 1 New therapeutic strategies tested by human hepatocyte chimeric mice

n	Drug or cell	Strategy	Reference
1	Interferon alpha 2b	Activation of antiviral genes	Kneteman *et al.*[65]
	BILN-2061	NS3-4A protease inhibition	
	HCV371	NS5B polymerase inhibition	
2	Modified BID	Induction of apoptosis	Hsu *et al.*[66]
3	Serine palmitoyltransferase inhibitor	Disruption of lipid raft	Umehara *et al.*[67]
4	Lymphoblastoid interferon alpha	Activation of antiviral genes	Hiraga *et al.*[14]
5	Amphipathic DNA polymers	Blocking viral entry	Matsumura *et al.*[60]
6	Sec-butyl-analogue of HCV-371	NS5B polymerase inhibition	LaPorte *et al.*[68]
7	HCV796	NS5B polymerase inhibition	Kneteman *et al.*[69]
8	Liver allograft-derived lymphocyte	Adoptive immunotherapy	Ohira *et al.*[70]
9	Telaprevir	NS3-4A protease inhibition	Kamiya *et al.*[71]

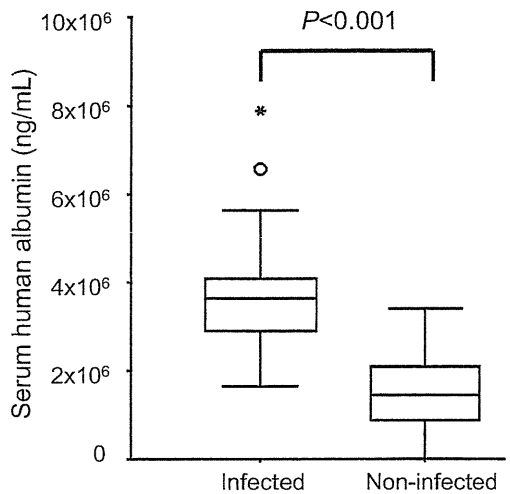

Figure 3 Human albumin levels in mice used in the hepatitis C virus (HCV) infection experiments. A total of 54 mice were injected with HCV positive serum samples containing 5×10^5 virus particles. A total of 24 mice became persistently positive for HCV-RNA, but 30 mice did not. Serum human albumin levels 2 weeks after human hepatocyte transplantation were compared between infected and non-infected mice.

CD81,[58] polyclonal human immunoglobulin directed to a similar strain,[59] and amphipathic DNA polymers.[60] Notably, the presence of broadly neutralizing antibodies to HCV that protect against heterologous viral infection has been reported, suggesting the possibility of a prophylactic vaccine against HCV.[61]

With respect to evasion of the virus against the innate immune response, altered intrahepatic expression profiles in the early phase of infection is of particular interest. The chimeric mice model is ideal for such studies; cross-hybridization of mouse and human can be avoided by careful experimental procedures.[62] Microarray analysis of livers of HCV infected and non-infected mice showed transcriptional activation of genes related to innate immune response, lipid metabolism, endoplasmic reticulum (ER) stress and apoptosis in HCV-infected mice.[63,64] The HCV infected mouse model is particularly useful for the study of newly developed HCV agents. The effect of recently developed chemicals and a unique therapy using intrahepatic lymphocytes have been shown using this model (Table 1). However, none of these therapies have yet been able to completely eradicate HCV from mice. It is noteworthy that ultra-rapid cardiotoxicity has been reported with the protease inhibitor BILN 2061 in the uPA/scid mice, but not in scid mice, implicating involvement of the uPA transgene.[72] Care should therefore be taken in interpreting the results obtained by this model.

Conclusion

Development of a small animal model using human hepatocyte chimeric mice has enabled us to study key aspects of HBV and HCV biology. The characteristic feature of the absence of human immune cells is suitable for studying viral replication and observing changes occurring in liver cells during viral infection, such as the innate immune response and cellular stress and metabolic responses. The model is also useful for studying the effect of drugs without the influence of cytokines and cytotoxic T lymphocytes. Nonetheless, the model is insufficient to study carcinogenesis of hepatitis viruses, because non-parenchymal cells in mouse liver are of mouse origin and do not support inflammation and fibrosis, which are probably closely related to carcinogenesis. The lack of human immune cells also limits the study of inflammation and immunity. Furthermore, the availability of human hepatocytes is limited. Despite these limitations, the current model shows great potential as a mouse model for the study of hepatitis viruses. Development of a small animal model with or without human immunity using stem cells or iPS cells would be an ideal model in the future.

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Research Paper

Efficacy of Lamivudine or Entecavir on Acute Exacerbation of Chronic Hepatitis B

Tatsuo Kanda[1*]✉, Masami Shinozaki[2*], Hidehiro Kamezaki[1], Shuang Wu[1], Shingo Nakamoto[1], Makoto Arai[1], Keiichi Fujiwara[1], Nobuaki Goto[2], Fumio Imazeki[1] and Osamu Yokosuka[1]

1. Department of Medicine and Clinical Oncology, Chiba University, Graduate School of Medicine, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan.
2. Department of Medicine, Numazu City Hospital, Numazu, Shizuoka 410-0302, Japan.

* These authors contributed equally to this article.

✉ Corresponding author: Tatsuo Kanda, MD, PhD, Associate Professor, Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8677, Japan. Tel.: +81-43-226-2086; Fax: +81-43-226-2088; E-mail: kandat-cib@umin.ac.jp

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Abstract

Background/Aims: Spontaneous acute exacerbation of chronic hepatitis B virus (HBV) infection occasionally occurs in its natural history, sometimes leading rapidly to fatal hepatic failure. We compared the effects of lamivudine (LAM) with those of entecavir (ETV) treatments in acute exacerbation of chronic hepatitis B with 500 IU/L or higher alanine aminotransferase (ALT) levels.

Methods: Thirty-four patients with acute exacerbation were consecutively treated with LAM /ETV. Their clinical improvements were compared.

Results: Among LAM-treated and ETV-treated patients, none showed a reduction of <1 log IU/mL in HBV DNA after 1 or 3 months of treatment. Initial virological response, defined as a reduction of 4 log IU/mL in HBV DNA at 6 months, with LAM and ETV, respectively, was 83.3% and 100%. One LAM patient developed hepatic encephalopathy, but all patients in both groups survived. Twelve months after treatment, 41.6% of 24 LAM group patients switched to another drug or added adefovir to their treatment due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change drugs.

Conclusions: ETV appears to be as effective as LAM in the treatment of patients with acute exacerbation of chronic hepatitis B. Clinicians should carefully start to treat these patients as soon as possible.

Key words: acute exacerbation, ALT, entecavir, HBV, lamivudine

INTRODUCTION

Chronic hepatitis B infection is associated with the development of hepatocellular carcinoma [1]. Infection with hepatitis B virus (HBV) also leads to wide a spectrum of liver injury, including acute, self-limited infection, fulminant hepatitis, and chronic hepatitis with progression to cirrhosis and liver failure, as well as to an asymptomatic chronic carrier state [2, 3].

Reactivation of hepatitis B is a well-characterized syndrome marked by the abrupt reappearance or rise of HBV DNA in the serum of a patient with previously inactivated or resolved HBV infection [4]. Reac-

tivation is often spontaneous, but can also be triggered by cancer chemotherapy and immune suppression. Spontaneous acute exacerbation of chronic hepatitis B infection is seen with a cumulative probability of 15-37% after 4 years of follow-up [5]. Prognosis is generally poor in HBV carriers with spontaneous acute exacerbation together with high alanine aminotransferase (ALT) levels, jaundice, and liver failure [4, 6, 7]. This condition has been defined as acute-on-chronic liver failure according to a recent Asia-Pacific consensus recommendation [8]. Acute exacerbation occasionally leads to a critical scenario, meaning that clinicians need to treat this condition immediately.

Lamivudine (LAM) is a reverse-transcriptase inhibitor of viral DNA polymerase with an excellent profile of safety and tolerability, causing inhibition of viral replication, and it is approved for antiviral treatment of hepatitis B patients [9, 10]. LAM suppresses serum HBV DNA values in up to 98% of patients within a median period of 4 weeks, leading to aminotransferase normalization, increased hepatitis B e antigen (HBeAg) seroconversion rate, and improvement of histological parameters [11, 12]. A study from Taiwan showed that LAM had a survival benefit and was effective for patients with baseline bilirubin levels below 20 mg/dL [7].

Entecavir (ETV), a deoxyguanosine analogue, is a potent and selective inhibitor of HBV replication; its *in vitro* potency is 100- to 1,000-fold greater than that of LAM, and it has a selectivity index (concentration of drug reducing the viable cell number by 50% [CC_{50}]/concentration of drug reducing viral replication by 50% [EC_{50}]) of ~8,000 [13, 14]. At present, the Japanese national health insurance system approves ETV as the first-line therapy for chronic hepatitis B, although some patients are treated with standard interferon-alfa. ETV is a nucleoside analogue (NUC) belonging to a new subgroup, cyclopentane [15], and it has been shown to be highly effective in suppressing HBV replication to an undetectable level and normalizing ALT, although NUCs do not eradicate the virus. ETV develops less resistance than LAM.

We undertook a retrospective study to compare the efficacy of LAM with that of ETV in the reduction of HBV DNA levels and associated improvement in disease severity and biochemical recovery in patients with acute exacerbation together with higher ALT levels due to HBV reactivation.

MATERIALS AND METHODS

Patients

A retrospective analysis of LAM/ETV-treated chronic hepatitis B patients at Chiba University Hospital and Numazu City Hospital, Japan, between May 2003 and December 2009 was performed. The inclusion criteria were: acute exacerbation of chronic hepatitis B characterized by an elevation of ALT level ≥ 500 IU/L along with HBV DNA ≥ 4.5 log IU/mL presenting in a patient with diagnosed chronic liver disease. The exclusion criteria were: acute hepatitis B, superinfection with other viruses (hepatitis E, A, D, or C), other causes of chronic liver failure [16, 17], coexistent hepatocellular carcinoma, portal thrombosis, coexistent renal impairment, pregnancy, coinfection with human immunodeficiency virus (HIV), or patients who had received a previous course of NUC treatment. This retrospective study protocol conforms to the ethical guidelines of the 1975 Declaration of Helsinki as reflected in *a priori* approval by the Ethics Committee of Chiba University, Graduate School of Medicine [18].

Baseline assessment of patients

Retrospectively collected data included patient demographics, clinical findings, all laboratory variables including virological tests and abdominal ultrasound. HBsAg, HBeAg, anti-HBe antibody and immunoglobulin M (IgM) anti-HBc antibody were determined by ELISA (Abbott, Chicago, IL, USA) or CLEIA (Fujirebio, Tokyo, Japan) [19]. HBV genotype was determined from patients' sera by ELISA (Institute of Immunology, Tokyo, Japan) as reported by Usuda et al [20]. HBV DNA was measured by Roche Amplicor™ PCR assay (detection limits: 2.6 log IU/mL; Roche Diagnostics, Tokyo, Japan).

Definitions

Primary antiviral treatment failure was defined as a reduction of < 1 log IU/mL in HBV DNA after 3 months of therapy. Initial virological response (IVR) was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

Follow-up

Clinical assessment and routine investigations were done every 15 days or every month for at least 6 months. HBV DNA measurements were repeated monthly.

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2010 for Windows™ 7 and StatView 5 (SAS Institute Inc, Cary, NC). Continuous variables were expressed as mean ± standard deviation and were compared by two-factor analysis of variance (ANOVA) and two-way repeated measures ANOVA. Categorical variables were compared by Chi-square

test. Baseline was taken as the date when the first dose of LAM/ETV was administered. Statistical significance was considered at a *P*-value < 0.05.

RESULTS

Patients

Between May 2003 and December 2009, 34 patients with spontaneous acute exacerbation of chronic hepatitis B, with ALT levels ≥ 500 IU/mL and treated with LAM or ETV, were consecutively enrolled and retrospectively analyzed. 24 (70.5%) were treated with LAM at 100 mg daily and 10 (29.4%) were treated with ETV at 0.5 mg daily. All patients were followed for at least 6 months. Mean follow-up in the LAM and ETV groups was 55.5 ± 25.4 and 16.5 ± 9.9 months, respectively.

Baseline characteristics

Baseline characteristics in the two patient groups were similar **(Table 1)**. Median age was 37 (21-73) years and 79.4% were men. One patient of the LAM group developed hepatic encephalopathy, but recovered. All patients in both groups survived. At admission, the serological profile showed HBsAg positivity in all 34 (100%); 22 (64.7%) were HBeAg positive. The median HBV DNA level was 7.4 log IU/mL in the LAM group and 7.9 log IU/mL in the ETV group **(Table 1)**.

Table 1 Demographic, Clinical, and Laboratory Variables of Patients at Entry.

Parameters	Total Patients (N=34)	LAM (N=24)	ETV (N=10)	*P*-value
Age (years)	37 (21-73)	37 (21-73)	39 (24-67)	NS
Male (%)	27 (79.4)	18 (75)	9 (90)	NS
Cirrhosis (+/-)	2/32	2/22	0/10	NS
ALT (IU/L)	986 (523-2,450)	995 (523-2,450)	1,046 (523-2,140)	NS
T. Bil (mg/dL)	2.0 (0.8-22.0)	2.4 (0.8-20.6)	1.6 (1.9-22.0)	NS
PT (%)	83 (24-121)	81.5 (24-119)	83.6 (35-121)	NS
HBeAg (+/-)	22/12	18/6	4/6	NS
HBV DNA (log IU/mL)	7.6 (4.8-8.7)	7.4 (5.2-8.7)	7.9 (4.8-8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in HBV DNA of total patients

LAM significantly reduced HBV DNA levels from baseline 7.24 log IU/mL to 3.27 log IU/mL at 1 month ($P < 0.001$), to 2.21 log IU/mL at 3 months ($P < 0.001$), and to 1.53 log IU/mL at 6 months ($P < 0.001$). ETV also significantly reduced HBV DNA levels from baseline 7.56 log IU/mL to 3.12 log IU/mL at 1 month ($P < 0.001$), to 2.14 log IU/mL at 3 months ($P < 0.001$), and to 1.77 log IU/mL at 6 months ($P < 0.001$). There were no differences in HBV DNA levels from baseline to 6 months between the two groups. None with primary antiviral treatment failure was identified in either group. There were no significant differences in IVR between the two groups **(Figure 1)**.

Reduction in ALT levels of total patients

LAM significantly reduced ALT levels from baseline 1,130 IU/mL to 102 ($P < 0.001$) at 1 month, to 28.6 ($P < 0.001$) at 3 months, and to 23.1 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,210 IU/mL to 117 ($P < 0.001$) at 1 month, to 25 ($P < 0.001$) at 3 months, and to 24.4 ($P < 0.001$) at 6 months. There were no differences in ALT levels from baseline to 6 months between the two groups **(Figure 2)**.

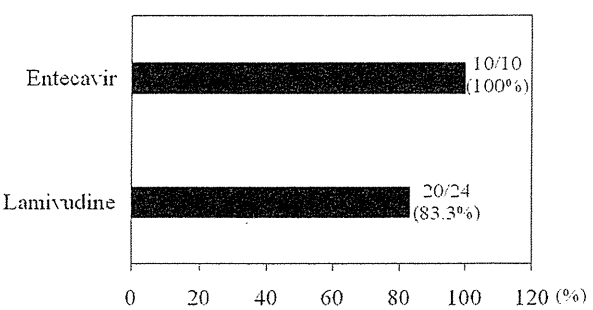

Figure 1 Initial virological response (IVR). IVR was defined as a reduction of ≥ 4 log IU/mL in HBV DNA after 6 months of therapy [21].

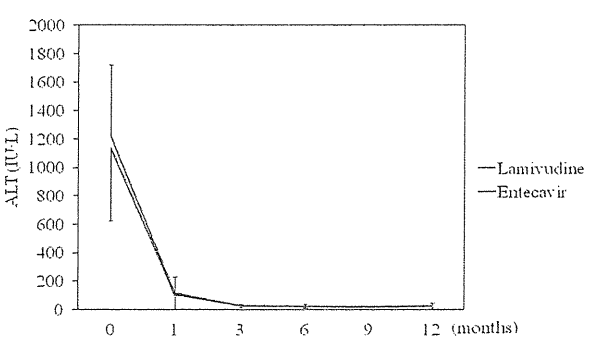

Figure 2 Efficacy of lamivudine and entecavir for ALT levels. Lamivudine (N=24) vs. entecavir (N=10); data are shown as mean ± SD.

Reduction in HBV DNA of HBeAg-positive patients

It has been demonstrated that the levels of HBV DNA in the HBeAg-positive phase were generally higher than those in the ant-HBe-positive phase [19, 22]. HBeAg positivity is also associated with HBV viremia and increased ALT levels in HIV/HBV co-infected patients [23]. Next, we compared the response to LAM or ETV in 18 or 4 HBeAg-positive patients, respectively **(Table 2)**. LAM significantly reduced HBV DNA levels from baseline 7.52 log IU/mL to 3.35 log IU/mL ($P < 0.001$) at 1 month, to 2.38 log IU/mL ($P < 0.001$) at 3 months, and to 1.55 log IU/mL ($P < 0.001$) at 6 months. ETV also significantly reduced HBV DNA levels from baseline 8.42 log IU/mL to 3.87 log IU/mL ($P < 0.001$) at 1 month, to 2.90 log IU/mL ($P < 0.001$) at 3 months, and to 2.22 log IU/mL ($P < 0.001$) at 6 months. There were no differences in HBV DNA levels from baseline to 6 months between the two groups. Primary antiviral treatment failure was not observed in either group. Four patients in the LAM group did not achieve IVR.

Table 2 Demographic, Clinical, and Laboratory Variables of HBeAg-positive Patients at Entry.

Parameters	Total Patients (N=22)	LAM (N=18)	ETV (N=4)	*P*-value
Age (years)	34.5 (21-51)	36.5 (21-51)	30 (24-33)	NS
Male (%)	18 (81.8)	14 (77.7)	4 (100)	NS
Cirrhosis (+/-)	1/21	1/17	0/4	NS
ALT (IU/L)	1,030 (523-2,450)	1,990 (523-2,450)	1,363 (980-1,620)	NS
T. Bil (mg/dL)	1.75 (0.8-20.6)	2.0 (0.8-20.6)	1.5 (1.0-18.7)	NS
PT (%)	77 (24-119)	73.6 (24-119)	95.0 (44.1-113)	NS
HBeAg (+)	22	18	4	
HBV DNA (log IU/mL)	7.6 (5.5- 8.8)	7.6 (5.5- 8.7)	8.6 (7.6- 8.7)	NS

LAM, lamivudine; ETV, entecavir; ALT, alanine aminotransferase; T. BIL, total bilirubin; PT, prothrombin time; NS, statistically not significant.

Reduction in ALT levels of HBeAg-positive patients

LAM significantly reduced ALT levels from baseline 1,150 IU/mL to 84 ($P < 0.001$) at 1 month, to 27.5 ($P < 0.001$) at 3 months, and to 22.0 ($P < 0.001$) at 6 months. ETV also significantly reduced ALT levels from baseline 1,460 IU/mL to 230 ($P = 0.0038$) at 1 month, to 22.2 ($P = 0.0016$) at 3 months, and to 24.0 ($P = 0.0016$) at 6 months. At 1 month after treatment, the ALT levels of the LAM groups were lower than those of the ETV group ($P < 0.0001$) **(Figure 3)**. During follow-up periods, 10 and 1 sero-converters of HBeAg to anti-HBe antibody phase were seen in 18 LAM-treated and in 4 ETV-treated patients, respectively.

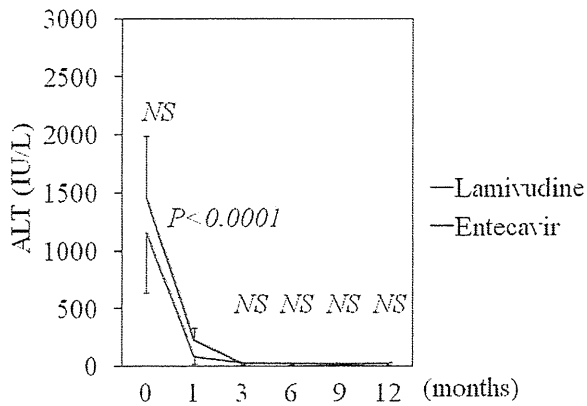

Figure 3 Efficacy of lamivudine and entecavir for ALT levels in HBeAg-positive patients. Lamivudine (N=18) vs. entecavir (N=4); data are shown as mean ± SD.

Safety

No patient stopped taking medications. Twelve months after treatment, 10 of 24 patients (41.6%) in the LAM group switched from LAM to ETV (n=4) or added adefovir (n=6) due to the emergence of LAM-resistant mutants. On the other hand, patients receiving ETV did not need to change their medication.

DISCUSSION

The present study compared the use of NUCs, LAM and ETV, for the treatment of acute exacerbation of chronic hepatitis B. The results clearly showed significant benefits of a rapid reduction of HBV DNA levels, compared with untreated patients in a previous report [4].

It was reported that ETV treatment is associated with increased short-term mortality in patients with severe acute exacerbation of chronic hepatitis B, but that it achieves better virological response in the long run [24]. We used LAM or ETV for patients with acute exacerbation of chronic hepatitis B presenting with ALT ≥ 500 IU/L in the present study. The effects of LAM on HBV DNA levels were the same as those of ETV (Figure 1). But the effects of LAM on ALT levels after 1 month were stronger than those of ETV in HBeAg-positive patients **(Figure 3)**. In spite of the limited number of these patients, the effects were possibly related to immunomodulating activities of LAM [25]. The patients' prognoses were more favorable than in the previous report [4]. This might have