Fig. 1. Phylogenetic tree of the entire nucleotide sequences constructed by the neighbor-joining method using the present four isolates (*), HBV/B isolates and other HBV genotype (A, C, D, E, F, G, and H) isolates retrieved from DDBJ/GenBank.

G1896A in precore region, A1762T and G1764A in core promoter region were in accord with those of previous reports [Sato et al., 1995]. Similarly, amino acid analysis showed Ile97Leu and Pro130Non-Pro in the core region and Trp28Stop in the precore region as previously reported [Kosaka et al., 1991; Aye et al., 1994].

Divergences of the Entire Genome and Amino Acids Between the Present Isolates and HBV/B2 Isolates From GenBank

Divergences of the entire genome sequence of the present four isolates with other Asian HBV/B2 isolates registered on GenBank are shown in Table II. Among the sequences registered on GenBank, AY121245 and AB073834 from Vietnam showed low divergences with them, 1.7%. As for registered isolates from patients with fulminant hepatitis, X97850 showed divergences of 3.2%. We compared the two consensus sequences of the nucleotides and amino acids derived from AB302943 and X97850 (fulminant hepatitis) versus the isolates from AB121245 and AB073834 (non-fulminant hepatitis). Only one different nucleotide (nt 1,504) was revealed in the P and X region, but they were not located in either promoter or enhancer regions. Nevertheless, it was recognized that most of the 95 isolates (HBV genome length 3,215 nucleotides) belonged to B2 registered on Genbank showed that nt 1,504 was not C (83 isolates were G, just 2 isolates were C, and 10 were others). Similarly, as for the amino acids, aa 44 in the X region was Ala for isolates from fulminant hepatitis, but it was not common for non-fulminant hepatitis (66 isolates were Val, just 2 isolates were Ala, and 27 isolates were others). In contrast to it, aa 805 in the P region showed no mutation.

DISCUSSION

We encountered five cases of fulminant hepatitis induced by HBV acute infection that occurred during 8 months in 2000–2001. All had been referred to our hospital within a short period. They had had no sexual contact, had not abused illegal drugs, nor had had contact with foreigners. According to the retrospective investigations, it was revealed that they had not had previous contact with each other, and that the only thing they had in common was that they had seen the same physician as out-patients. It was thought to be very difficult to be determined their infectious routes, since not all of them had had chances such as intravenous injections in those days. Two of the patients had just been administered drugs for hyperlipidemia or hypertension. However, the later investigations revealed that they had be drawn blood tests with syringes which were not disposable, although it was impossible to prove their infectious source as the usage of this syringe at the time of investigation. However, the inadequate handling of autoclaving apparatus could possibly cause insufficient sterilization of glass syringes, which were still utilized at several clinics at that time. If the syringes, which were routinely used to draw blood, were contaminated with HBV, it is difficult to prevent fatal acute hepatitis, since the infected patients will never develop symptoms until large amount of infected hepatocytes will be collapsed. Thus, preventive medial intervention is extremely difficult in this setting once infection is established. They were all rather aged (average 67 years old), which might have worsened their clinical prognosis. As for the therapy, liver transplantation was not common in Japan in those days. Moreover, the worsening of the clinical course was so rapid that it would have been difficult to find suitable donors for them. We administered intensive therapy, including plasma exchange, continuous hemodiafiltration, and methyl-prednisolone administration (initially 1,000 mg/day and tapered). Four patients could not be rescued.

As for the clinical diagnosis, we confirmed that the patients suffered from fulminant hepatitis due to acute HBV infection as follows. First, none of them had a family history of hepatitis or prior liver diseases. Second, the serum tests of the present five patients showed anti IgM class anti-HBc positive, compatible with acute HBV infection.

Serologically, all of them were anti-HBe positive, similar to some previous reports from Japan [Kosaka et al., 1991; Omata et al., 1991; Sato et al., 1995; Inoue et al., 2006].

In this study, we at first determined the HBV partial nucleotide sequences in the S region (nt 278–646). Four sequences of them were in complete accord, and one isolate (FH-4) showed just one nucleotide difference. This result suggested the possibility of a common infectious route; this was based on just 11.5% of the entire HBV 3,215 nucleotides (369/3,125). Thereafter, we tried to determine the entire nucleotide sequences.

We determined the entire nucleotide sequences of the HBV from four patients, and their divergences showed 0–0.3%.

These results could imply that the patients were infected with the same HBV isolate. On the phylogenetic tree analysis, they belonged to HBV/B, subgroup B2. HBV/B is known to be widespread in Asian countries, but as we previously reported, HBV/B could be divided into five minor groups on the phylogenetic tree, whose geographical distances and genetic distances are well correlated. The HBV strain found in the present patients was not a typical strain in Japan. The typical HBV subgenotype in Japan is B1, and the B2 isolate is mainly prevalent in China, Vietnam, and Taiwan [Norder et al., 2004].

We could not determine the entire HBV nucleotide sequences in one patient (FH-5), possibly because of the presence of some mutations in the primer regions we used.

It was previously reported that patients with HBV/B2 have a rapidly progressive and severe clinical course [Sugauchi et al., 2002]. As for chronic infection, Sugauchi reported that Japanese patients with HBV/B2 showed HBe antigen more frequently than those with HBV/B1 [Orito et al., 2001].

As for acute HBV infection, it is not clear whether the genotypes of HBV show clinical differences. It is still controversial whether specific HBV genotypes are more often associated with fulminant hepatitis [Gandhe et al., 2003; Chen et al., 2004]. Further examination concerning the relation between the HBV subgenotypes and their clinical manifestations is needed.

The precore stop mutation (G1896A) and core promoter mutations (A1762T and G1764A) have been reported to have an association with fulminant hepatitis in Japan [Sato et al., 1995], but not common in other countries, such as the US and Germany [Laskus et al., 1993; Sterneck et al., 1998]. The association of G1896A, A1762T, and G1764A with fulminant hepatitis induced by HBV infection is controversial [Carman et al., 1989; Aritomi et al., 1998; Friedt et al., 1999; Yuasa et al., 2000; Chen et al., 2003; Kao et al., 2003]. A universal, specific genomic mutational pattern associated with fulminant hepatitis has not been found [Sterneck et al., 1996].

The prevalence of the above mutations is known to differ according to the HBV genotype. A1762T and G1764A are common in HBV/C, but not in HBV/B [Chan et al., 1999]. In contrast to it, G1896A is common in HBV/B and HBV/C, but rare in the HBV genotype A [Stuyver et al., 2000]. The above discrepancy might derive from the distribution of the HBV genotypes and their virological characteristics, but further studies including in vitro studies will be needed.

As for previously known nucleotide mutations in HBV/B, our present isolates had G1896A, A1762T, and G1764A. They were in accord with those previously reported for fulminant hepatitis induced by acute HBV/B infection [Ozasa et al., 2006].

Additionally, we analyzed for the subgenotype. We compared the entire two consensus nucleotide and amino acid sequences for fulminant hepatitis (AB302942 and X97851) versus non-fulminant hepatitis (AF121245 and AB073834) among the isolates belonging to HBV/B2. We chose two isolates for the following reason. We should compare the HBV genome from patients with fulminant hepatitis and acute hepatitis, but isolates from acute hepatitis belonging to B2 have not been confirmed on Genbank. Therefore, we at first compared two consensus nucleotides from isolates related to fulminant hepatitis and other isolates without G1896A related to fulminant hepatitis by HBV/B. Thereafter, the difference of nt 1,504 in the P and X region and the difference of aa 44 in the X region were revealed.

Nucleotide 1,504 in the P and X region was not located in either the promoter or the enhancer regions. It is difficult to determine the significance of this difference, it was outstanding among the 95 isolates (HBV genome length 3,215 nucleotides) of B2 registered on Genbank. These might have some relation to fulminant hepatitis by isolates belonging to HBV/B2. As for amino acids, aa 44 in the X region was different between them, but it was not involved in essential part of X protein [Runkel et al., 1993].

The previously reported nucleotide mutations, C1653T and T1753M, were not detected in the present isolates [Kaneko et al., 1995; Sato et al., 1995]. Our results might indicate the possibility of a relationship between fulminant hepatitis induced by HBV/B and A1762T and G1764A.

A similar analysis for amino acid mutations showed Ile97Leu and Pro130Non-Pro in the core region and Trp28Stop in the precore region as previously reported [Kosaka et al., 1991; Aye et al., 1994]. In contrast, Met1 non-Met and Ser183Pro were absent.

These results could imply the strong relationship between fulminant hepatitis induced by HBV infection and some nucleotide/amino acid mutations in pre-C/C region of HBV, and partially those in P/X region of HBV.

We analyzed the entire nucleotide sequences of HBV/B from five patients with fulminant hepatitis, all of whom presented during a short period of time and showed rapidly worsening clinical courses. Virologically, the present HBV isolates may have the potential to lead infected patients to a fatal outcome. We need further studies, including in vitro studies of isolates, and comparison with other isolates detected from fulminant hepatitis patients in the future.

REFERENCES

Aritomi T, Yatsuhashi H, Fujino T, Yamasaki K, Inoue O, Koga M, Kato Y, Yano M. 1998. Association of mutations in the core promoter and precore region of hepatitis virus with fulminant and severe acute hepatitis in Japan. J Gastroenterol Hepatol 13: 1125–1132.

Aye TT, Uchida T, Becker SO, Hirashima M, Shikata T, Komine F, Moriyama M, Arakawa Y, Mima S, Mizokami M, et al. 1994. Variations of hepatitis B virus precore/core gene sequence in acute and fulminant hepatitis B. Dig Dis Sci 39:1281–1287.

Carman WF, Jacyna MR, Hadziyannis S, Karayiannis P, McGarvey MJ, Makris A, Thomas HC. 1989. Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. Lancet 2:588–591.

Chan HL, Hussain M, Lok AS. 1999. Different hepatitis B virus genotypes are associated with different mutations in the core promoter and precore regions during hepatitis B e antigen seroconversion. Hepatology 29:976–984.

Chen Y, Michitaka K, Matsubara H, Yamamoto K, Horiike N, Onji M. 2003. Complete genome sequence of hepatitis B virus (HBV) from a patient with fulminant hepatitis without precore and core promoter mutations: Comparison with HBV from a patient with acute hepatitis infected from the same infectious source. J Hepatol 38: 84–90.

Chen CH, Lee CM, Lu SN, Wang JH, Tung HD, Hung CH, Chen WJ, Changchien CS. 2004. Comparison of clinical outcome between patients continuing and discontinuing lamivudine therapy after biochemical breakthrough of YMDD mutants. J Hepatol 41:454–461.

Chisari FV, Ferrari C. 1995. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 13:29–60.

Feray C, Gigou M, Samuel D, Reyes G, Bernuau J, Reynes M, Bismuth H, Brechot C. 1993. Hepatitis C virus RNA and hepatitis B virus DNA in serum and liver of patients with fulminant hepatitis. Gastroenterology 104:549–555.

Friedt M, Gerner P, Lausch E, Trubel H, Zabel B, Wirth S. 1999. Mutations in the basic core promotor and the precore region of hepatitis B virus and their selection in children with fulminant and chronic hepatitis B. Hepatology 29:1252–1258.

Gandhe SS, Chadha MS, Arankalle VA. 2003. Hepatitis B virus genotypes and serotypes in western India: Lack of clinical significance. J Med Virol 69:324–330.

Inoue K, Ogawa O, Yamada M, Watanabe T, Okamoto H, Yoshiba M. 2006. Possible association of vigorous hepatitis B virus replication

with the development of fulminant hepatitis. J Gastroenterol 41: 383–387.

Kaneko M, Uchida T, Moriyama M, Arakawa Y, Shikata T, Gotoh K, Mima S. 1995. Probable implication of mutations of the X open reading frame in the onset of fulminant hepatitis B. J Med Virol 47: 204–208.

Kao JH, Chen PJ, Lai MY, Chen DS. 2003. Basal core promoter mutations of hepatitis B virus increase the risk of hepatocellular carcinoma in hepatitis B carriers. Gastroenterology 124:327–334.

Kikuchi K, Niitsuma H, Ishii M, Cervantes JG, Hong S, Ojima T, Suzuki C, Kobayashi T, Ueno Y, Kobayashi K, Shimosegawa T, Toyota T. 2000. Genoepidemiology and its relationship to clinical features in patients infected chronically with hepatitis B virus (HBV). Hepatol Res 17:43–55.

Kobayashi M, Suzuki F, Akuta N, Hosaka T, Sezaki H, Yatsuji H, Yatsuji H, Kobayashi M, Suzuki Y, Arase Y, Ikeda K, Watahiki S, Iwasaki S, Miyakawa Y, Kumada H. 2007. Loss of hepatitis B surface antigen from the serum of patients with chronic hepatitis treated with lamivudine. J Med Virol 79:1472–1477.

Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshiba M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, et al. 1991. Fulminant hepatitis B: Induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. Gastroenterology 100:1087–1094.

Laskus T, Persing DH, Nowicki MJ, Mosley JW, Rakela J. 1993. Nucleotide sequence analysis of the precore region in patients with fulminant hepatitis B in the United States. Gastroenterology 105: 1173–1178.

Lee WM. 1997. Hepatitis B virus infection. N Engl J Med 337:1733–1745.

Liaw YF, Chen YC, Sheen IS, Chien RN, Yeh CT, Chu CM. 2004. Impact of acute hepatitis C virus superinfection in patients with chronic hepatitis B virus infection. Gastroenterology 126:1024–1029.

Nagasaki F, Niitsuma H, Cervantes JG, Chiba M, Hong S, Ojima T, Ueno Y, Bondoc E, Kobayashi K, Ishii M, Shimosegawa T. 2006. Analysis of the entire nucleotide sequence of hepatitis B virus genotype B in the Philippines reveals a new subgenotype of genotype B. J Gen Virol 87:1175–1180.

Niitsuma H, Ishii M, Saito Y, Miura M, Kobayashi K, Ohori H, Toyota T. 1995. Prevalence of precore-defective mutant of hepatitis B virus in HBV carriers. J Med Virol 46:397–402.

Norder H, Hammas B, Lofdahl S, Courouce AM, Magnius LO. 1992. Comparison of the amino acid sequences of nine different serotypes of hepatitis B surface antigen and genomic classification of the corresponding hepatitis B virus strains. J Gen Virol 73:1201–1208.

Norder H, Courouce AM, Coursaget P, Echevarria JM, Lee SD, Mushahwar IK, Robertson BH, Locarnini S, Magnius LO. 2004. Genetic diversity of hepatitis B virus strains derived worldwide: Genotypes, subgenotypes, and HBsAg subtypes. Intervirology 47: 289–309.

Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. 1988. Typing hepatitis B virus by homology in nucleotide sequence: Comparison of surface antigen subtypes. J Gen Virol 69:2575–2583.

Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. 1991. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 324:1699–1704.

Orito E, Mizokami M, Sakugawa H, Michitaka K, Ishikawa K, Ichida T, Okanoue T, Yotsuyanagi H, Iino S. 2001. A case-control study for clinical and molecular biological differences between hepatitis B viruses of genotypes B and C. Japan HBV Genotype Research Group. Hepatology 33:218–223.

Ozasa A, Tanaka Y, Orito E, Sugiyama M, Kang JH, Hige S, Kuramitsu T, Suzuki K, Tanaka E, Okada S, Tokita H, Asahina Y, Inoue K, Kakumu S, Okanoue T, Murawaki Y, Hino K, Onji M, Yatsuhashi H, Sakugawa H, Miyakawa Y, Ueda R, Mizokami M. 2006. Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. Hepatology 44:326–334.

Perrillo RP, Aach RD. 1981. The clinical course and chronic sequelae of hepatitis B virus infection. Semin Liver Dis 1:15–25.

Runkel L, Fischer M, Schaller H. 1993. Two-codon insertion mutations of the HBx define two separate regions necessary for its trans-activation function. Virology 197:529–536.

Sagnelli E, Coppola N, Messina V, Di Caprio D, Marrocco C, Marotta A, Onofrio M, Scolastico C, Filippini P. 2002. HBV superinfection in hepatitis C virus chronic carriers, viral interaction, and clinical course. Hepatology 36:1285–1291.

Sakugawa H, Nakasone H, Nakayoshi T, Kawakami Y, Yamashiro T, Maeshiro T, Kinjo F, Saito A. 2001. Correlation between serum transaminase activity and virus load among patients with chronic liver disease type B. Hepatol Res 21:159–168.

Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. 1995. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 122:241–248.

Shan H, Niitsuma H, Nagasaki F, Cervantes J, Ojima T, Ueno Y, Kobayashi K, Ishii M, Shimosegawa T. 2002. Analysis of the entire nucleotide sequence of hepatitis B virus: Characteristics of HBeAg-positive asymptomatic carriers, HBeAb positive asymptomatic carriers and patients with liver cirrhosis. Hepatol Res 23: 251.

Sterneck M, Gunther S, Santantonio T, Fischer L, Broelsch CE, Greten H, Will H. 1996. Hepatitis B virus genomes of patients with fulminant hepatitis do not share a specific mutation. Hepatology 24:300–306.

Sterneck M, Kalinina T, Gunther S, Fischer L, Santantonio T, Greten H, Will H. 1998. Functional analysis of HBV genomes from patients with fulminant hepatitis. Hepatology 28:1390–1397.

Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. 2000. A new genotype of hepatitis B virus: Complete genome and phylogenetic relatedness. J Gen Virol 81: 67–74.

Sugauchi F, Orito E, Ichida T, Kato H, Sakugawa H, Kakumu S, Ishida T, Chutaputti A, Lai CL, Ueda R, Miyakawa Y, Mizokami M. 2002. Hepatitis B virus of genotype B with or without recombination with genotype C over the precore region plus the core gene. J Virol 76: 5985–5992.

Yuasa R, Takahashi K, Dien BV, Binh NH, Morishita T, Sato K, Yamamoto N, Isomura S, Yoshioka K, Ishikawa T, Mishiro S, Kakumu S. 2000. Properties of hepatitis B virus genome recovered from Vietnamese patients with fulminant hepatitis in comparison with those of acute hepatitis. J Med Virol 61:23–28.

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RAPID COMMUNICATION

Pegylated interferon plus ribavirin for genotype Ib chronic hepatitis C in Japan

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Abstract

AIM: To evaluate the efficacy of pegylated interferon α-2b (peg-IFNα-2b) plus ribavirin (RBV) therapy in Japanese patients with chronic hepatitis C (CHC) genotype Ib and a high viral load.

METHODS: One hundred and twenty CHC patients (58.3% male) who received peg-IFNα-2b plus RBV therapy for 48 wk were enrolled. Sustained virological response (SVR) and clinical parameters were evaluated.

RESULTS: One hundred (83.3%) of 120 patients completed 48 wk of treatment. 53 patients (44.3%) achieved SVR. Early virological response (EVR) and end of treatment response (ETR) rates were 50% and 73.3%, respectively. The clinical parameters (SVR *vs* non-SVR) associated with SVR, ALT (108.4 IU/L *vs* 74.5 IU/L, $P = 0.063$), EVR (76.4% *vs* 16.4%, $P < 0.0001$), adherence to peg-IFN (≥ 80% of planned dose) at week 12 (48.1% *vs* 13.6%, $P = 0.00036$), adherence to peg-IFN at week 48 (54.7% *vs* 16.2%, $P < 0.0001$) and adherence to RBV at week 48 (56.1% *vs* 32.1%, $P = 0.0102$) were determined using univariate analysis, and EVR and adherence to peg-IFN at week 48 were determined using multivariate analysis. In the older patient group (> 56 years), SVR in females was significantly lower than that in males (17% *vs* 50%, $P = 0.0262$). EVR and adherence to Peg-IFN were demonstrated to be the main factors associated with SVR.

CONCLUSION: Peg-IFNα-2b plus RBV combination therapy demonstrated good tolerability in Japanese patients with CHC and resulted in a SVR rate of 44.3%. Treatment of elderly female patients is still challenging and maintenance of adherence to peg-IFNα-2b is important in improving the SVR rate.

Key words: Chronic hepatitis C; Pegylated interferon; Ribavirin

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INTRODUCTION

In Japan, annual mortality due to liver cancer exceeds 30 000 and 75% of liver cancer is associated with hepatitis C virus (HCV) infection[1]. The combination of pegylated interferon (peg-IFN) plus ribavirin (RBV) is one of the most effective therapies for chronic hepatitis C (CHC), and the effect of this combination is reported to be higher than conventional interferon[2,3]. However, the majority of Japanese CHC patients are infected with HCV genotype Ib and have a high viral load, and treatment with conventional interferon has its difficulties[4]. CHC patients in Japan tend to be older than CHC patients in other countries therefore, problems such as a higher incidence of liver cancer and lower tolerability to treatment have been observed[4,5]. The HCV strain and the efficacy of interferon treatment vary between races and countries[6,7]. Identification of the factors associated with treatment efficacy is extremely important, however, few studies involving large populations have reported on the treatment of Japanese CHC patients with pegylated interferon alpha-2b (peg-IFNα-2b) plus RBV[8,9]. In this study, we evaluated the efficacy and safety of peg-IFNα-2b plus RBV therapy in CHC genotype Ib patients with a high viral load. This treatment became available in Japan for health insurance approved treatment from December 2004. In addition, we attempted to identify predictive factors for treatment outcome.

MATERIALS AND METHODS

Study population

One hundred and thirty CHC genotype Ib patients with a high viral load, who received peg-IFNα-2b plus RBV therapy in our hospital or our affiliated institutions between December 2005 and November 2006 were enrolled in this study. The diagnosis of CHC was based on the following criteria; HCV antibody positive, HCV-RNA positive and elevation of serum alanine aminotransferase (ALT) activity (> 35 IU/L) within 6 mo of screening. Exclusion criteria were leucopenia [white blood cell (WBC) count < 3000/μL], neutropenia [neutrophil (ne) count <1500/μL], thrombocytopenia [platelet (PLT) count < 90000/μL], anemia [hemoglobin (Hb) < 12 g/dL], cirrhosis, creatinine clearance < 50 mL/min, uncontrolled mental disorder, severe heart or lung disease, or autoimmune disease. The study was approved by the ethical committee of Tohoku University according to the Declaration of Helsinki. All patients gave written informed consent before enrollment.

Treatment regimen

The patients received peg-IFNα-2b (Pegintron®; Schering-Plough, Kenilworth, NJ, USA) at a dosage of 1.5 mg/kg every week subcutaneously for 48 wk. Daily RBV (Rebetol®, Schering-Plough) was given orally for 48 wk and the dosage was adjusted according to weight (600 mg for ≤ 60 kg, 800 mg for 60 to 80 kg, 1000 mg for > 80 kg). Blood samples were obtained every four weeks and were analyzed for biochemical parameters including ALT and HCV RNA levels. The HCV genotype was determined using a kit. HCV genotype was determined by PCR using a mixed primer set derived from the nucleotide sequences of the NS5 region. HCV RNA levels were measured by quantitative RT-PCR (Amplicor, Roche Diagnostic Systems, CA, USA). HCV RNA negativity was evaluated by qualitative RT-PCR (Amplicor, Roche), which has a higher sensitivity than the quantitative method. The lower limit of the assay in the quantitative method was 5 KIU/mL (equivalent to 5000 copies/mL) and was 50 IU/mL (equivalent to 50 copies/mL) in the qualitative method. Early virological response (EVR) was defined as undetectable HCV RNA after 12 wk. Sustained virological response (SVR) was defined as undetectable HCV RNA at 24 wk after completion of treatment.

Statistical analysis

Fisher's exact test and the Mann-Whitney *U* test were used to evaluate the parameters [age, sex, weight, body mass index (BMI), EVR, peg-IFN adherence, RBV adherence, HCV RNA, ALT, WBC, Hb, and PLT] to determine SVR. Quantitative data were divided into two groups using the median to examine the differences. We conducted multivariate analysis using binary logistic regression on the parameters which achieved statistical significance ($P < 0.05$) using univariate analysis. All analyses were performed using a statistical software package (StatView-J version 5.0, SAS Institute Inc. Cary, NC, USA).

RESULTS

Patient characteristics

The details of clinical background, blood biochemistry and virological data on the CHC patients who received peg-IFNα-2b plus RBV therapy are shown in Table 1. Seventy of 120 patients (58.3%) were male, and 50 patients (41.7%) were female. The mean age was 54.8 years, and the median age was 56 years. The mean age of males was 54.1 years, and the mean age of females was 55.8 years. The median BMI was 23.6. Seventy seven patients (64.2%) had no previous history of IFN treatment and 41 patients (34.2%) had been treated with IFN previously. Of these previously treated patients, 8 were null-responders (patients who did not achieve a virological or biochemical response during IFN treatment), 15 were relapsers, and 18 patients had no available virological response.

Treatment efficacy

One hundred of 120 patients (83.3%) completed 48 wk of treatment and 24 wk of follow up. Using intention to treat (ITT) analysis, 53 patients (44.3%) achieved SVR. The rate of EVR was 50%. Response rate at the end of treatment was 73.3%. The transition rate of HCV RNA negativity with time is shown in Figure 1. Patients discontinued treatment due to depression in 3, neutropenia in 3, retinopathy in 2, anemia in 1,

Table 1 Clinical characteristics of patients at baseline (mean ± SE)

No. of patients	120
Sex, *n* (%)	
Male	70 (58.3)
Female	50 (41.7)
Age (median, range, yr)	54.8 ± 0.98 (56, 27-75)
Male	54.1 ± 1.39 (55.5, 29-72)
Female	55.8 ± 1.33 (56, 27-75)
Weight	62.1 ± 1.09 (61.4, 35.0-99.8)
Body mass index (median, range, kg)	23.7 ± 0.32 (23.6, 14.6-34.1)
Viral load (kIU kirocopies/mL)	1510 (120->5000)
ALT (median, range, IU/L)	89.4 ± 7.39 (67, 18-636)
WBC (median, range, /μL)	5083 ± 136.6 (4900, 2400-9000)
Hemoglobin (median, range, g/dL)	14.4 ± 0.12 (14.1, 11.8-17.2)
Platelet (median, range, × 10^3/μL)	163.1 ± 4.71 (162.5, 8.1-33.2)
Interferon treatment history, *n* (%)	
Present	41 (34.2)
Null-responder/relapser/unknown	8/15/18
Absent	77 (64.2)
Unknown	2 (1.6)

ALT: Alanine aminotransferase; WBC: White blood cell.

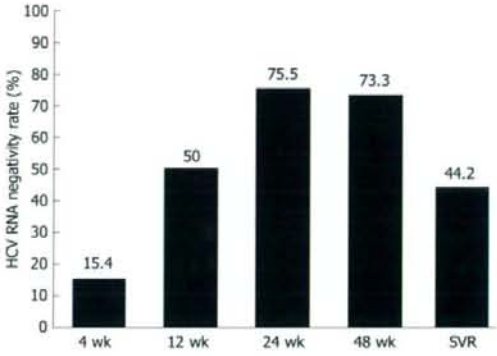

Figure 1 The transition rate of HCV RNA negativity with time.

cutaneous reaction in 1, palsy in 1, HSV infection in 1, and no response to treatment in 7.

Relationship between clinical parameters and SVR

The association between SVR rate and the baseline clinical parameters before treatment or treatment-related factors was examined using univariate analysis. The following baseline factors were analyzed: age, sex, BMI, HCV RNA level, ALT, WBC, Hb, and PLT. A summary of these results is shown in Table 2. The mean ALT level in patients who achieved SVR was 108.4 IU/L, which was significantly higher than the ALT level of 74.5 IU/L in the non-SVR group ($P = 0.0478$). The PLT level in patients in the SVR group was 1.73 ×10^5/μL, which was higher than the PLT level of 1.55 ×10^5/μL in the non-SVR group ($P = 0.063$). To determine the factors associated with treatment outcome, we examined the relationship between the SVR ratio and achievement of EVR or adherence to peg-IFN and RBV. A summary of these results is shown in Table 2 and Figure 2. As shown in Table 2, the ratio of patients who achieved EVR was

Table 2 Univariate analysis of association between sustained virological response (SVR) and influential factors (mean ± SE)

Factor	SVR patients (*n* = 53)	Non-SVR patients (*n* = 67)	*P*
Parameters before interferon treatment			
Age (yr)	52.5 ± 1.50	56.5 ± 1.26	0.0481
Sex (Male:Female)	35:18	35:32	0.1402
Body mass index	23.6 ± 0.48	23.8 ± 0.44	0.3611
Viral load (kirocopies/mL, median)	1500	1800	0.1963
ALT (IU/L)	108.4 ± 13.8	74.5 ± 7.04	0.0478
WBC (/μL)	5227 ± 201	4967 ± 186	0.2880
Hemoglobin (g/dL)	14.5 ± 0.18	14.3 ± 0.16	0.2352
Platelet (× 10^3/μL)	173 ± 7.7	155 ± 5.7	0.0630
Parameters associated with treatment			
EVR	42/51 (82.4%)	13/59 (22.0%)	< 0.0001
Cumulative exposure to peg-IFN			
12 wk (≥ 80%/< 80%)	38/41 (92.7%)	41/69 (68.3%)	0.0034
Overall (≥ 80%/< 80%)	35/41 (85.4%)	29/60 (48.3%)	0.0001
Cumulative exposure to RBV			
12 wk (≥ 80%/< 80%)	41/50 (82%)	44/63 (69.8%)	0.1882
Overall (≥ 80%/< 80%)	32/50 (64%)	25/63 (39.7%)	0.0138

ALT: Alanine aminotransferase; WBC: White blood cell; EVR: Early virological response; Peg-IFN: Pegylated interferon; RBV: Ribavirin.

Table 3 Multivariate analysis of association between sustained virological response and influential factors

Factor	Coefficient	χ^2	Odds Ratio (95% CI)	*P*
EVR (not achieved)	-2.725	19.325	0.066 (0.019-0.221)	< 0.0001
Cumulative exposure to peg-IFN				
Overall (≥ 80%)	2.392	6.600	10.934 (1.763-67.82)	0.0102
Constant	1.294			

EVR: Early virological response; Peg-IFN: Pegylated interferon.

significantly higher in the SVR group than in the non-SVR group ($P < 0.0001$). The SVR rate in patients who achieved EVR was 76.4%, and this was significantly higher than the SVR rate in the non-EVR group which was 16.4% ($P < 0.0001$). The ratio of patients who received 80% or more of the scheduled dose of peg-IFN or RBV was significantly higher in the SVR group than in the non-SVR group. The SVR rate in patients who received 80% or more of the scheduled dose of peg-IFN was 48.1% (12th wk) and 54.7% (overall). The SVR rate in patients who did not receive sufficient peg-IFN was 13.6% (12th wk) and 16.2% (overall), and these were significantly lower than the group who had good adherence. The group with adequate adherence to RBV (overall) showed an SVR rate of 56.1%, which was significantly higher than the SVR rate of 32.1% in the poor adherence group ($P = 0.0102$). For the factors which were determined as statistically significant by univariate analysis, we subsequently conducted multivariate analysis. The results of this analysis are shown in Table 3. Using binary logistic analysis, EVR and adherence to peg-IFN were determined to be independent predictive factors for SVR.

We examined a group of patients who were older than the median age (56 years). From the baseline factors obtained before treatment, sex was determined

Table 4 Univariate analysis of association between sustained virological response (SVR) and influential factors (mean ± SE)

Factor	SVR patients (n = 20)	Non-SVR patients (n = 37)	P
Parameters before interferon treatment			
Age (yr)	64.0 ± 0.71	63.8 ± 0.73	0.6637
Sex (male:female)	16:4	18:19	0.0262
Body mass index	23.3 ± 0.56	23.6 ± 0.53	0.3973
Viral load (kirocopies/mL)	1500	1800	0.3616
ALT (IU/L)	94.5 ± 31.1	76.6 ± 11.0	0.3038
WBC (/μL)	5119 ± 313	4832 ± 223	0.3798
Hemoglobin (g/dL)	14.1 ± 0.23	14.1 ± 0.19	0.8473
Platelet (× 10^3/μL)	173 ± 12.4	151 ± 7.9	0.1434
Parameters associated with treatment			
EVR	13/18 (72.2%)	7/32 (21.9%)	0.0008
Cumulative exposure to peg-IFN			
12 wk (≥ 80%/< 80%)	16/17 (94.1%)	20/33 (60.6%)	0.0183
Overall (≥ 80%/< 80%)	15/17 (88.2%)	14/33 (42.4%)	0.0023
Cumulative exposure to RBV			
12 wk (≥ 80%/< 80%)	14/20 (70%)	20/34 (58.8%)	0.5612
Overall (≥ 80%/< 80%)	8/20 (40%)	13/34 (38.2%)	> 0.9999

ALT: Alanine aminotransferase; WBC: White blood cell; EVR: Early virological response; Peg-IFN: Pegylated interferon; RBV: Ribavirin.

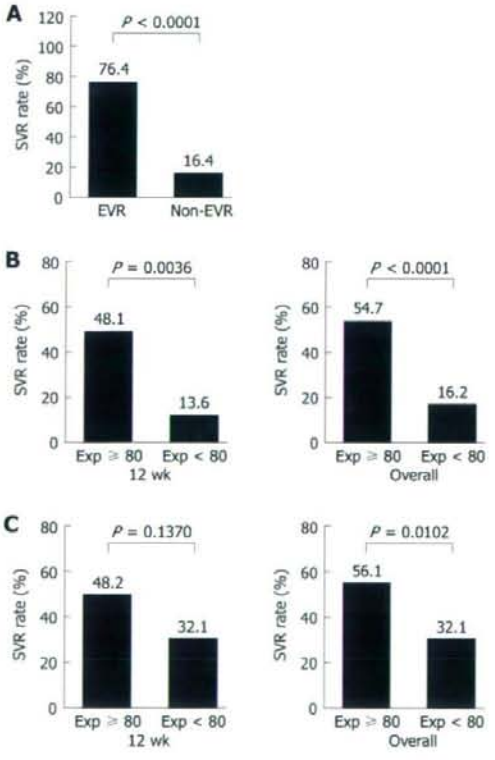

Figure 2 The clinical parameters associated with SVR rate using univariate analysis. A: The relationship between EVR and SVR rate; B: The relationship between cumulative exposure to peg-IFN and SVR rate; C: The relationship between cumulative exposure to RBV and SVR rate.

to be a parameter which may be associated with SVR (Table 4). The SVR rate in females was 17%, which was significantly lower than the SVR rate of 50% in males

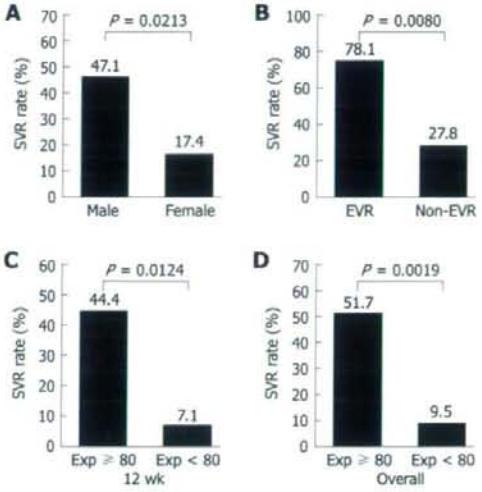

Figure 3 Clinical parameters in the older patient group (> 56 yr) associated with SVR rate using univariate analysis. A: The relationship between sex and SVR rate; B: The relationship between EVR and SVR. C and D: The relationship between cumulative exposure to peg-IFN and SVR.

(P = 0.0262) (Figure 3A). From the factors associated with treatment outcome, EVR and adherence to peg-IFN were demonstrated to be significant (Figure 3B). In particular, the SVR rate in the group with poor adherence to peg-IFN was 7.1% (1 of 14) at the 12th wk and was 9.5% (2 of 21) at the end of treatment. These rates were extremely low compared with the SVR rate of 44.4% (12th wk) and 51.7% (overall) in the group with good adherence to peg-IFN (Figure 3C and D).

DISCUSSION

One hundred of 130 patients completed peg-IFNα-2b plus RBV combination therapy in our hospital and related institutions. Treatment was discontinued in 13 patients (10.8%) due to adverse effects. The treatment showed good tolerability in Japanese patients. A study of peg-IFNα-2b plus RBV combination therapy in Caucasian and African American CHC patients reported a discontinuation rate of 21%[10]. Another study on Japanese CHC patients reported a 21% discontinuation rate[9]. Although we cannot compare these studies directly, it seems that tolerability in this study was satisfactory. At least in a clinical setting, peg-IFNα-2b plus RBV was well tolerated in our study of Japanese patients.

In this study, age, ALT level, EVR achievement, and adherence to Peg-IFN and RBV were associated with a high SVR rate using univariate analysis. After multivariate analysis, EVR and adherence to peg-IFN were demonstrated to be associated with SVR. Of the baseline factors assessed before treatment, age, sex, WBC, α-feto protein level, γ-glutamyl transpeptidase, and LDL-cholesterol have been reported to be associated with a high SVR rate following peg-IFNα-2b plus RBV therapy in CHC Japanese patients[8,11,12]. The results of this study were very similar to those of our

study. Davis *et al*[13] reported that EVR was considered to be associated with SVR in patients with CHC treated with IFN. As a result of this study, EVR was found to be one of the factors which most influenced SVR rate in Japanese patients treated with peg-IFNα-2b plus RBV combination therapy. In our study of older patients (older than the median), sex, EVR, and adherence to peg-IFN were associated with SVR rate. The SVR rate in older females was remarkably low at 17.4% compared to the SVR rate in all females included in the study which was 36.0% (data not shown).

Adherence to peg-IFN was found to influence the SVR rate as a treatment-related factor in this study. SVR rates were low in patients who did not receive 80% or more of the intended dose of peg-IFN. The effect of adherence to IFN on SVR has been reported previously[13-15]. In a study on peg-IFNα-2a/RBV therapy in patients with HCV genotype I, it was reported that the SVR rate in cases who had a reduction in RBV dosage before the 20th wk was remarkably low[15]. Furthermore, a reduction in RBV dosage and/or peg-IFNα-2a dosage after the 24th wk did not influence the SVR rate[15]. On the other hand, a study on African American patients with HCV genotype I reported that a reduction in peg-IFNα-2b dosage influenced the SVR rate more than a reduction in RBV dosage[14]. In the current study, adherence to RBV up to the 12th wk did not significantly influence the SVR rate, but overall adherence to RBV significantly influenced the SVR rate. Unlike the reports on Caucasian and African American patients, it may be that overall adherence to RBV is important in Japanese patients.

It was notable that adherence to peg-IFNα-2b significantly influenced SVR in this study. In the patients who did not receive 80% or more of the intended dose by the 12th wk, the SVR rate decreased markedly. Adherence to peg-IFNα-2b at the 12th wk may be critical in determining whether the treatment should be continued. It is often difficult to maintain adherence to peg-IFNα-2b simply to improve the SVR rate, because IFN dosage and the hematologic adverse effects of this drug are problematic[16,17]. Recently, a 72-wk treatment protocol for late virological responders was reported[18,19]. Further examination of the impact of prolonged administration in patients with poor adherence to peg-IFNα-2b is needed.

In conclusion, peg-IFNα-2b plus RBV combination therapy demonstrated good tolerability in Japanese patients with CHC, and resulted in a SVR rate of 44.3%. Treatment of older female patients and maintenance of adherence to peg-IFNα-2b are important factors in improving SVR rate.

COMMENTS

Background

Pegylated interferon α-2b (peg-IFNα-2b) plus ribavirin (RBV) is a standard treatment of chronic hepatitis C globally. However, the impact of this treatment in an ordinary clinical setting in Asian patients is still unclear.

Research frontiers

It is well documented that data from clinical practice is not comparable to those of clinical trials. This is believed to be derived from differences in recruited patients in phase II and III clinical trials and usual clinical settings (e.g. young *vs* elderly, highly motivated *vs* reluctant, *etc*).

Innovations and breakthroughs

The current study demonstrated that outcome is dependent on therapeutic adherence (> 80% of expected peg-IFN dosage). The overall treatment success [sustained virological response (SVR)] was 44.3%, almost equivalent to those in phase III clinical trials.

Applications

The total SVR rate was equivalent to clinical trials. The elderly, especially female patients showed a lower response to treatment. The reason for this is still unclear and future investigations are feasible in order to understand this observation.

Terminology

SVR indicates sustained virological response, which means sustained (more than 24 wk after treatment) viral clearance from the infected host.

Peer review

It is very important to describe the true clinical impact of global standard treatment in Asian races. Fortunately, the results were almost equivalent to those of other global regions. Although female patients seem to have a disadvantage with this treatment, these patients could have comparable results if adherance to both drugs is maintained.

REFERENCES

1 **Umemura T**, Kiyosawa K. Epidemiology of hepatocellular carcinoma in Japan. *Hepatol Res* 2007; **37** Suppl 2: S95-S100

2 **Manns MP**, McHutchison JG, Gordon SC, Rustgi VK, Shiffman M, Reindollar R, Goodman ZD, Koury K, Ling M, Albrecht JK. Peginterferon alfa-2b plus ribavirin compared with interferon alfa-2b plus ribavirin for initial treatment of chronic hepatitis C: a randomised trial. *Lancet* 2001; **358**: 958-965

3 **McHutchison JG**, Gordon SC, Schiff ER, Shiffman ML, Lee WM, Rustgi VK, Goodman ZD, Ling MH, Cort S, Albrecht JK. Interferon alfa-2b alone or in combination with ribavirin as initial treatment for chronic hepatitis C. Hepatitis Interventional Therapy Group. *N Engl J Med* 1998; **339**: 1485-1492

4 **Higuchi M**, Tanaka E, Kiyosawa K. Epidemiology and clinical aspects on hepatitis C. *Jpn J Infect Dis* 2002; **55**: 69-77

5 **Kiyosawa K**, Umemura T, Ichijo T, Matsumoto A, Yoshizawa K, Gad A, Tanaka E. Hepatocellular carcinoma: recent trends in Japan. *Gastroenterology* 2004; **127**: S17-S26

6 **He XS**, Ji X, Hale MB, Cheung R, Ahmed A, Guo Y, Nolan GP, Pfeffer LM, Wright TL, Risch N, Tibshirani R, Greenberg HB. Global transcriptional response to interferon is a determinant of HCV treatment outcome and is modified by race. *Hepatology* 2006; **44**: 352-359

7 **Luo S**, Cassidy W, Jeffers L, Reddy KR, Bruno C, Howell CD. Interferon-stimulated gene expression in black and white hepatitis C patients during peginterferon alfa-2a combination therapy. *Clin Gastroenterol Hepatol* 2005; **3**: 499-506

8 **Akuta N**, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Miyakawa Y, Kumada H. Prediction of response to pegylated interferon and ribavirin in hepatitis C by polymorphisms in the viral core protein and very early dynamics of viremia. *Intervirology* 2007; **50**: 361-368

9 **Hiramatsu N**, Kurashige N, Oze T, Takehara T, Tamura S, Kasahara A, Oshita M, Katayama K, Yoshihara H, Imai Y, Kato M, Kawata S, Tsubouchi H, Kumada H, Okanoue T, Kakumu S, Hayashi N. Early decline of hemoglobin can predict progression of hemolytic anemia during pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C. *Hepatol Res* 2008; **38**: 52-59

10 **Muir AJ**, Bornstein JD, Killenberg PG. Peginterferon alfa-2b and ribavirin for the treatment of chronic hepatitis C in blacks and non-Hispanic whites. *N Engl J Med* 2004; **350**: 2265-2271

11 **Akuta N**, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. Predictors of viral kinetics to peginterferon plus ribavirin combination therapy in Japanese patients infected with hepatitis C virus genotype 1b. *J Med Virol* 2007; **79**: 1686-1695

12 **Akuta N**, Suzuki F, Kawamura Y, Yatsuji H, Sezaki H, Suzuki Y, Hosaka T, Kobayashi M, Kobayashi M, Arase Y, Ikeda K, Kumada H. Amino acid substitutions in the hepatitis C virus core region are the important predictor of hepatocarcinogenesis. *Hepatology* 2007; **46**: 1357-1364

13 **Davis GL**, Wong JB, McHutchison JG, Manns MP, Harvey J, Albrecht J. Early virologic response to treatment with peginterferon alfa-2b plus ribavirin in patients with chronic hepatitis C. *Hepatology* 2003; **38**: 645-652

14 **McHutchison JG**, Manns M, Patel K, Poynard T, Lindsay KL, Trepo C, Dienstag J, Lee WM, Mak C, Garaud JJ, Albrecht JK. Adherence to combination therapy enhances sustained response in genotype-1-infected patients with chronic hepatitis C. *Gastroenterology* 2002; **123**: 1061-1069

15 **Shiffman ML**, Di Bisceglie AM, Lindsay KL, Morishima C, Wright EC, Everson GT, Lok AS, Morgan TR, Bonkovsky HL, Lee WM, Dienstag JL, Ghany MG, Goodman ZD, Everhart JE. Peginterferon alfa-2a and ribavirin in patients with chronic hepatitis C who have failed prior treatment. *Gastroenterology* 2004; **126**: 1015-1023; discussion 947

16 **Russo MW**, Fried MW. Side effects of therapy for chronic hepatitis C. *Gastroenterology* 2003; **124**: 1711-1719

17 **Soza A**, Everhart JE, Ghany MG, Doo E, Heller T, Promrat K, Park Y, Liang TJ, Hoofnagle JH. Neutropenia during combination therapy of interferon alfa and ribavirin for chronic hepatitis C. *Hepatology* 2002; **36**: 1273-1279

18 **Arase Y**, Ikeda K, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Kobayashi M, Suzuki F, Akuta N, Someya T, Kumada H. Efficacy of prolonged interferon therapy for patients with chronic hepatitis C with HCV-genotype 1b and high virus load. *J Gastroenterol* 2003; **38**: 158-163

19 **Arase Y**, Suzuki F, Tsubota A, Suzuki Y, Saitoh S, Kobayashi M, Akuta N, Someya T, Hosaka T, Kobayashi M, Sezaki H, Ikeda K, Kumada H. Sustained negativity for HCV-RNA over 24 or more months by long-term interferon therapy correlates with eradication of HCV in patients with hepatitis C virus genotype 1b and high viral load. *Intervirology* 2004; **47**: 19-25

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Case report

Analysis of the full-length genome of hepatitis B virus in the serum and cerebrospinal fluid of a patient with acute hepatitis B and transverse myelitis☆

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Abstract

Although many extrahepatic manifestations have been described in patients with acute or chronic hepatitis B, there are few reports about neurological disorders. We describe a 55-year-old man who contracted acute hepatitis B virus (HBV) infection and transverse myelitis. His neurological findings were gradually reduced along with the recovery from hepatitis. The cerebrospinal fluid (CSF) was revealed to be positive for HBsAg and HBV DNA. Full-length sequences of HBV in his serum and CSF were determined, and it was revealed that these two isolates had mutations at nucleotide (nt) 1762/1764 in the core promoter region and nt 1896 in the precore region. They were identical to each other except for two ambiguous codes at nt 2020 and 2631 in the CSF isolate. After cloning of the amplicons, substitutions at nt 2020 and 2631 were found in 6 (38%) of the 16 CSF clones. One clone of the 6 CSF clones had an additional substitution at nt 2119. These substitutions were not found in 16 serum clones. The presence of HBV clones unique to CSF suggests that HBV was a possible causative agent of the myelitis.

Keywords: Hepatitis B virus; Acute hepatitis B; Transverse myelitis; Cerebrospinal fluid; Sequence analysis; Full-length genome

1. Introduction

Although hepatocytes are the primary locus of infection of hepatitis B virus (HBV), it was reported that 16% of patients with chronic HBV infection have extrahepatic clinical manifestations (Cacoub et al., 2005). Many of these are thought not due to extrahepatic infection of HBV, but to autoimmune and related phenomena. However, increasing evidence suggests the possibility of HBV infection of non-hepatic cells (Mason et al., 1993; Neurath et al., 1990; Seifer et al., 1990; Umeda et al., 2005). Although HBV DNA has been found in the cerebrospinal fluid (CSF) of infected individuals (Pao et al., 1987), there is little detailed information concerning HBV infection in the central nervous system (CNS). The relation between HBV and neurological disorders is poorly understood. Recently, a case of acute hepatitis B that occurring soon after the acute onset of transverse myelitis was seen in our hospital, suggesting a causal relationship. Because such a case has never been reported previously, evaluation of HBV markers in CSF and sequence analysis of HBV DNA in the serum and CSF were undertaken.

2. Case report

A 55-year-old Japanese male was admitted to our hospital with complaints of numbness in the perianal area, weakness

☆ The nucleotide sequence data reported in this study have been assigned GenBank/EMBL/DDBJ accession numbers AB298720 and AB298721.

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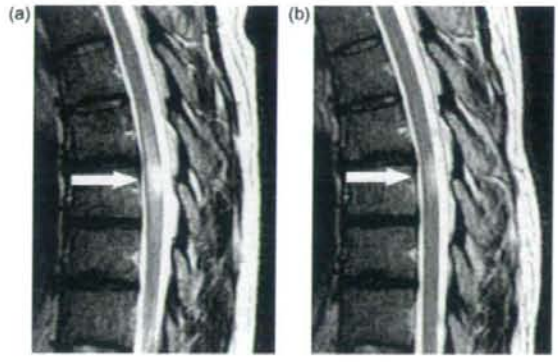

Fig. 1. (a) T2-weighted MRI of the thoracic spinal cord before the onset of acute hepatitis. A high intensity lesion at the Th7-8 level with spinal swelling is observed. (b) T2-weighted MRI 2 months after discharge. The lesion of the spinal cord is diminished.

and numbness of both lower extremities, and difficulty in urination. These symptoms began 2 months previously; peaked in severity after 1 month; then began slowly improving for the next month before admission. T2-weighted magnetic resonance imaging (MRI) of the thoracic (Th) spinal cord showed a high intensity area at the Th7-8 level (Fig. 1a) consistent with transverse myelitis. CSF obtained on hospital day 3 contained 10 monocytes/μL and 69 mg/dL of protein. At this time, liver function tests revealed elevation of alanine aminotransferase (ALT) (491 IU/L). His serum contained hepatitis B surface antigen (HBsAg) and was weakly positive for immunoglobulin M (IgM) antibody to hepatitis B core (HBc) (anti-HBc IgM). Both hepatitis B e antigen (HBeAg) and antibodies to HBeAg (HBeAb) were negative, and the HBV DNA concentration in plasma determined by transcription-mediated amplification (TMA) assay was over 8.6 log genome-equivalent. Ten days later, anti-HBc IgM increased and he was diagnosed with acute hepatitis B. The infection was presumed to have been acquired during sexual contact with a prostitute in Korea 3 months previously. He had no other risk factors for HBV infection. Without antiviral therapy, his liver function recovered from a peak ALT of 2632 IU/L and peak total bilirubin of 14.0 mg/dL. Hepatic encephalopathy, coagulopathy or ascites did not occur. His neurological findings continued to gradually improve along with liver function tests. After discharge on hospital day 30, HBsAg and HBV DNA became to be undetectable and HBsAb has turned to be positive. Two months after discharge, MRI showed that the spinal cord lesion had diminished (Fig. 1b).

To clarify the relationship between HBV infection and myelitis the CSF was evaluated for evidence of HBV infection. The CSF sample obtained in our hospital was positive for HBsAg and contained 1.6×10^6 copies/mL of HBV DNA measured by real-time PCR in the LightCycler system (Roche Diagnostics, Mannheim, Germany) as reported previously (Jardi et al., 2001). The concentration of HBV DNA in the serum at the time of sampling of CSF was 7.7×10^{10} copies/mL and had been 1.1×10^{11} copies/mL the previous day. Anti-HBc IgM in the CSF was not detectable by in-house ELISA (Tsatsralt-Od et al., 2006).

Because red blood cells in the CSF was not determined during the routine examination, the extent of blood contamination could not be evaluated. To determine the origin of the HBV in the CSF, we compared full-length sequences of HBV in serum and CSF. Using the serum and CSF samples obtained on the same day, amplification of the entire HBV genome was performed by methods similar to those described previously (Shibayama et al., 2005). Briefly, two overlapping regions (nt 190–1775 and nt 1673–3215/1–228) of HBV DNA were amplified by nested PCR with PrimeSTAR HS DNA Polymerase (TaKaRa Bio Inc., Shiga, Japan) and primers designed within conserved areas of the HBV genomes of the eight genotypes (A to H) (Arauz-Ruiz et al., 2002; Norder et al., 1994; Okamoto et al., 1988; Stuyver et al., 2000). The amplification products were sequenced directly or after cloning on both strands. The phylogenetic tree constructed based on the full genome sequences of HBV revealed that the HBV isolates from serum (BAJT2006-1S) and CSF (BAJT2006-1C) belonged to genotype C (Fig. 2). These isolates had mutations at nucleotides (nt) 1762/1764 (A1762T/G1764A) in

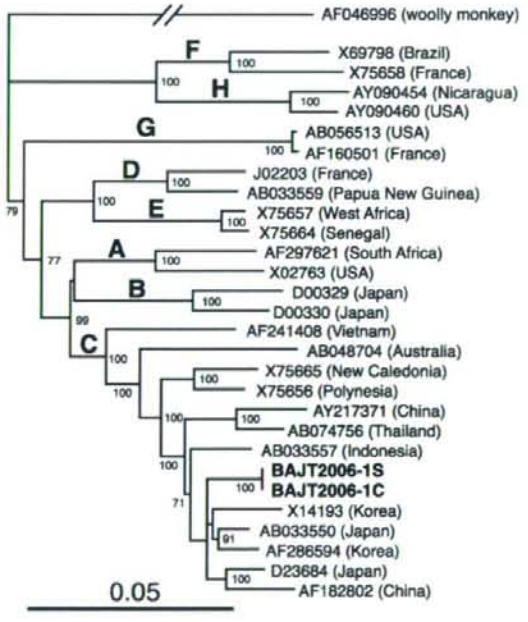

Fig. 2. A phylogenetic tree constructed based on the entire nucleotide sequences of 28 HBV isolates, using a woolly monkey HBV isolate (AF046996) as an out group. In addition to the two isolates obtained from serum (BAJT2006-1S) and CSF (BAJT2006-1C), 26 reported HBV isolates of genotypes A to H were included for comparison. The previously reported isolates are indicated with the accession no. followed by the name of the country where it was isolated. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1,000 resamplings.

(a)

```
BAJT2006-1S  2001 CCTCTGCTCTGTATCGGGAGGCCTTAGAGTCTCCGGAACATTGTTCACCT  2050
BAJT2006-1C  2001 --------------------R------------------------------  2050
GT C wild    2001 ----------------------------------------------------  2050

BAJT2006-1S  2601 CTTACAGTTAATGAAAAAAGGAGATTAAAATTAATTATGCCTGCTAGGTT  2650
BAJT2006-1C  2601 ----------------------------W-----------------------  2650
GT C wild    2601 ----------------------------------------------------  2650
```

(b)

Sample	No. of clones	Nucleotide no.		
		2020	2119	2631
Serum	16	G	A	T
CSF	10	G	A	T
CSF	5	A	A	A
CSF	1	A	G	A

Fig. 3. (a) Alignment of partial sequences (nt 2001–2050 and 2601–2650) of BAJT2006-1S and BAJT2006-1C. Sequence of wild type HBV of genotype C (GT C wild) was included for comparison. R is G or A, and W is A or T. (b) Nucleotides at nt 2020, 2119 and 2631 after cloning of BAJT2006-1S (serum) and BAJT2006-1C (CSF). The numbers of clones which had each indicated nucleotide were shown.

the core promoter region and nt 1896 (G1896A) in the precore region, and were identical to each other except for two ambiguous codes at nt 2020 (R: G or A) and nt 2631 (W: A or T) in BAJT2006-1C (Fig. 3a). After cloning of the amplicons of nt 1673–3215/1–228 recovered from serum and CSF, G to A substitutions at nt 2020 (G2020A) and T to A at nt 2631 (T2631A) were found in 6 (38%) of the 16 CSF clones (Fig. 3b). One clone of the six CSF clones with G2020A and T2631A had an additional substitution at nt 2119 of A to G (A2119G). None of the 16 serum clones had these substitutions. G2020A and A2119G did not change the amino acid sequences, whereas T2631A converted amino acid 109 of Leu to Ile in HBV polymerase.

3. Discussion

Because there are few reports about HBV in the CNS, it remains unknown whether HBV is merely present in the CNS after blood stream dissemination or can replicate in the CNS of HBV-infected individuals, especially in patients with acute HBV infection. In this report, HBsAg and HBV DNA were detected in the CSF of a patient with acute hepatitis B and transverse myelitis. Evidence that this did not represent contamination of CSF with blood was provided by sequence analysis of blood and CSF HBV clones. The HBV clones obtained from CSF were heterogeneous and 38% (6/16) of the clones were different from homogeneous clones obtained from the serum. Although the virological significance of the substitutions at nt 2020, 2119, and 2631 is unclear, HBV with these substitutions might favor replication in the CNS. The presence of HBV clones unique to CSF suggests that the HBV DNA in the CSF was not from contamination of by blood, and that HBV was possibly the cause of the myelitis. Additionally, the HBV isolates recovered from serum and CSF had in common A1762T/G1764A and G1896A, which are known to be associated with fulminant hepatitis B (Kosaka et al., 1991; Liang et al., 1991; Omata et al., 1991; Sato et al., 1995). The strains with these mutations have heightened replicative activity (Baumert et al., 1996; Hasegawa et al., 1994). Although the patient in this study did not develop fulminant hepatitis, the concentration of HBV in the serum in the acute phase reached 1.1×10^{11} copies/mL. The high replicative potential of this patient's HBV, in addition to nt substitutions in the CSF clones, may have contributed its ability to replicate in the CNS and cause myelitis.

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References

Arauz-Ruiz P, Norder H, Robertson BH, Magnius LO. Genotype H: a new Amerindian genotype of hepatitis B virus revealed in Central America. J Gen Virol 2002;83:2059–73.

Baumert TF, Rogers SA, Hasegawa K, Liang TJ. Two core promotor mutations identified in a hepatitis B virus strain associated with fulminant hepatitis result in enhanced viral replication. J Clin Invest 1996;98:2268–76.

Cacoub P, Saadoun D, Bourliere M, Khiri H, Martineau A, Benhamou Y, Varastet M, Pol S, Thibault V, Rotily M, Halfon P. Hepatitis B virus genotypes and extrahepatic manifestations. J Hepatol 2005;43:764–70.

Hasegawa K, Huang J, Rogers SA, Blum HE, Liang TJ. Enhanced replication of a hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. J Virol 1994;68:1651–9.

Jardi R, Rodriguez F, Buti M, Costa X, Cotrina M, Valdes A, Galimany R, Esteban R, Guardia J. Quantitative detection of hepatitis B virus DNA in serum by a new rapid real-time fluorescence PCR assay. J Viral Hepat 2001;8:465–71.

Kosaka Y, Takase K, Kojima M, Shimizu M, Inoue K, Yoshiba M, Tanaka S, Akahane Y, Okamoto H, Tsuda F, Miyakawa Y, Mayumi M. Fulminant hepatitis B: induction by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. Gastroenterology 1991;100:1087–94.

Liang TJ, Hasegawa K, Rimon N, Wands JR, Ben-Porath E. A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. N Engl J Med 1991;324:1705–9.

Mason A, Wick M, White H, Perrillo R. Hepatitis B virus replication in diverse cell types during chronic hepatitis B virus infection. Hepatology 1993;18:781–9.

Neurath AR, Strick N, Sproul P, Ralph HE, Valinsky J. Detection of receptors for hepatitis B virus on cells of extrahepatic origin. Virology 1990;176:448–57.

Norder H, Courouce AM, Magnius LO. Complete genomes, phylogenetic relatedness, and structural proteins of six strains of the hepatitis B virus, four of which represent two new genotypes. Virology 1994;198:489–503.

Okamoto H, Tsuda F, Sakugawa H, Sastrosoewignjo RI, Imai M, Miyakawa Y, Mayumi M. Typing hepatitis B virus by homology in nucleotide

sequence: comparison of surface antigen subtypes. J Gen Virol 1988;69: 2575–83.

Omata M, Ehata T, Yokosuka O, Hosoda K, Ohto M. Mutations in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis. N Engl J Med 1991;324:1699–704.

Pao CC, Wu SY, Hung IJ, Ng KT, Liaw YF, Lo SJ. Intra blood–cerebrospinal fluid-barrier detection of hepatitis B virus. Biochem Biophys Res Commun 1987;146:452–5.

Sato S, Suzuki K, Akahane Y, Akamatsu K, Akiyama K, Yunomura K, Tsuda F, Tanaka T, Okamoto H, Miyakawa Y, Mayumi M. Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. Ann Intern Med 1995;122:241–8.

Seifer M, Heermann KH, Gerlich WH. Replication of hepatitis B virus in transfected nonhepatic cells. Virology 1990;179:300–11.

Shibayama T, Masuda G, Ajisawa A, Hiruma K, Tsuda F, Nishizawa T, Takahashi M, Okamoto H. Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. J Med Virol 2005;76:24–32.

Stuyver L, De Gendt S, Van Geyt C, Zoulim F, Fried M, Schinazi RF, Rossau R. A new genotype of hepatitis B virus: complete genome and phylogenetic relatedness. J Gen Virol 2000;81:67–74.

Tsatsralt-Od B, Takahashi M, Endo K, Buyankhuu O, Baatarkhuu O, Nishizawa T, Okamoto H. Infection with hepatitis A, B, C, and delta viruses among patients with acute hepatitis in Mongolia. J Med Virol 2006;78:542–50.

Umeda M, Marusawa H, Seno H, Katsurada A, Nabeshima M, Egawa H, Uemoto S, Inomata Y, Tanaka K, Chiba T. Hepatitis B virus infection in lymphatic tissues in inactive hepatitis B carriers. J Hepatol 2005;42:806–12.

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Case Report

A case of HIV co-infected with hepatitis B virus precore/core deletion mutant treated by entecavir

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We report a case of a HIV and hepatitis B virus (HBV)-co-infected patient to whom entecavir (ETV) was administered initially before the notification regarding the potential mutagenesis effect on HIV against the nucleoside analog. Since initial evaluations indicated the advanced stage of chronic hepatitis B and preserved numbers of peripheral CD4+ lymphocytes without the manifestation of immunodeficiency, priority was given to the management of HBV. We started HBV therapy with ETV at a dose of 0.5 mg daily without using any HIV drugs. The viral loads of both HBV and HIV-1 decreased gradually during the 5 months following the initial administration of ETV. HBV was well controlled by the gradual replacement of ETV with highly-active antiretroviral therapy against HIV with a regimen including atazanavir, emtricitabine, and tenofovir. HBV was genotyped as A2 with the quasispecies pool consisting of the −1G precore/core deletion mutant strain.

Key words: co-infection, entecavir, hepatitis B virus, HIV

INTRODUCTION

ENTECAVIR (ETV), AN analog of 2′-deoxyguanosine, is regarded as an effective inhibitor of hepatitis B virus (HBV) and also inhibits HIV-1 replication both *in vitro* and *in vivo*. It selects for mutations (such as the M184V mutation) in HIV-1 reverse transcriptase, leading to lamivudine (LMV) and emtricitabine (FTC) resistance. Previous guidelines recommended ETV as the first-line treatment for patients with HIV-1 and HBV co-infection who do not require anti-HIV therapy.[1,2] In April, 2007, the US Food and Drug Administration (FDA) recommended avoiding the usage of ETV for HBV- and HIV-co-infected patients since the inhibitory effect of ETV against HIV may develop resistant HIV against nucleoside therapy.[3] A novel HBV precore/core deletion mutant referred to as the −1G deletion was identified in HIV-co-infected patients.[4] The −1G deletion is located in a homopolymeric string of guanosine nucleotides between HBV core nucleotides 185 and 190 and introduces a frameshift that terminated the coding sequence of the HBV core and precore genes, which leads logically to the production of the corresponding truncated core protein. The −1G deletion mutant was associated with a high HBV viral DNA concentration in these cases.

CASE REPORT

A 39-YEAR-OLD man visited us in December 2006 for the management of his hepatitis B. He also had carried HIV without any symptoms. The results of the initial examination of his serum were as follows: aspartate aminotransferase, 115 IU/l; alanine aminotransferase, 62 IU/l; total bilirubin, 2.2 mg/dL; prothrombin time (%, international normalized ratio), 32.2%, 2.22; number of peripheral lymphocytes, 1550/μL; CD4/CD8, 30.5%/60.0% (0.5); number of platelets, 50×10^3/μl; hepatitis B surface (HBs) antigen, positive; anti-HBs antibody, negative; hepatitis B e (HBe) antigen, positive; anti-HBe antibody, negative;

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The sequence of hepatitis B virus reported in this article has been deposited in the DDBJ/EMBL/GenBank databases under accession number AB353732.
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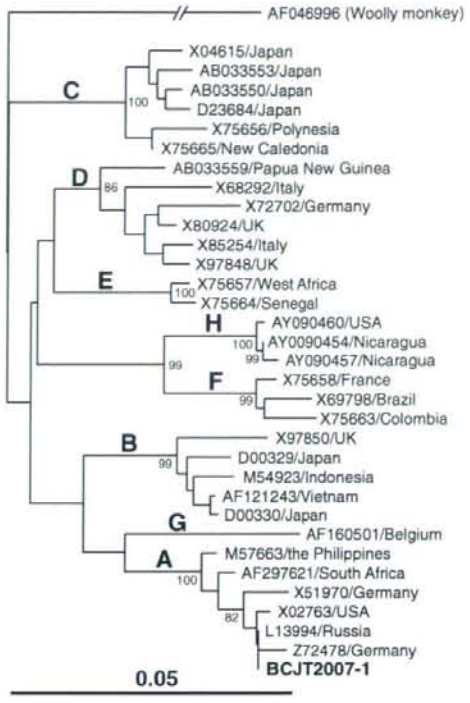
(a)



(b)

	Nt and aa sequences of nt 2063-2101	No.clones	
Genotype A wild	CTCAGGCAAGCCATTCTCTGCTGGGGGGAATTGATGACT LeuArgGlnAlaIleLeuCysTrpGlyGluLeuMetThr	Pre-ETV (23 Jan)	Post-ETV (3 Apr)
-1G deletion	CTCAGGCAAGCCATTCTCTGCTGGGGG/AATTGATGACT LeuArgGlnAlaIleLeuCysTrpGly Asn***	4/10	0/10

Figure 1 (a) Phylogenetic tree constructed based on the partial nucleotide sequence of the S gene (396 nt) of 33 hepatitis B virus (HBV) isolates, using a woolly monkey HBV isolate (AF046996) as an outgroup. In addition to the isolate obtained from the present case (BCJT2007-1), 32 representative HBV isolates of genotypes A–H were included for comparison. Previously reported isolates are indicated with the accession number followed by the name of the country where it was isolated. Bootstrap values are indicated for the major nodes as a percentage of the data obtained from 1000 resamplings. (b) Sequence alignments and numbers of the subcloned HBV –1G precore/core deletion mutant from 10 clones at a pair of time points are shown. Wild sequence was identical to the reference sequence of genotype A HBV listed in DDBJ/EMBL/GenBank (accession number: Z72478). ***, stop codon.

HBV–DNA, 7.6 log (10) copies/mL; HBV genotype, A2 (Fig. 1a); immunoglobulin G 4670 mg/dL; antinuclear antibody, 320×; antismooth muscle antibody, 160×; HIV-1 RNA 23×10^3 copies/mL; and antihepatitis C antibody, negative. A further analysis of the HBV sequence revealed a –1G precore/core deletion mutant along with the wild strain (Fig. 1b). Liver biopsy was not performed because of impaired coagulation. We considered his liver function to be seriously impaired, probably due to the chronic hepatitis B or autoimmune hepatitis. With regard to HIV, antiviral therapy was not necessary because of the preserved numbers of $CD4^+$

lymphocytes and low titers of HIV RNA. Thus there were several concerns to be considered for the management of his hepatitis. Our decision was to start anti-HBV therapy with the oral administration of a nucleoside analog, ETV, which appeared to be the best option at that time in January 2007. His serum number of HBV-DNA and HIV RNA decreased gradually along with an improvement of liver function during the 5 months from the initial therapy with ETV at the dose of 0.5 mg daily (Fig. 2). Based on the FDA recommendation at the end of April 2007, highly-active antiretroviral therapy (HAART) against HIV and HBV, which was the only alternative option, was employed. HAART, with a combination of 200 mg atazanavir, 300 mg tenofovir (TDF), and 200 mg FTC was started in early in July 2007 with the patient's consent. In the mutational analysis, we did not detect M204V/I of HBV.

Laboratory testing procedures are performed as follows.The titers of serum HBs antigen, HBs antibody, HBe antigen, and the HBe antibody, or a number of copies of HBV-DNA and HIV RNA were evaluated by chemiluminescent immunoassay, enzyme-linked immunosorbent assay, or polymerase chain reaction (PCR) and reverse transcription PCR on consignment with commercially-available diagnostic kits. Nucleic acids were extracted from 50 μL serum with a QIAamp DNA blood mini kit (QIAGEN, Tokyo, Japan). For HBV genotyping and the mutational analysis of the core promoter and precore region, a nucleic acid sample was subjected to nested PCR with primer sets based on the well-conserved sequence in the S gene region and corresponding regions. A phylogenetic tree was constructed by the neighbor-joining method based on the 396-nt sequence identified by direct sequence with the ABI PRISM 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA) for genotyping as described previously.[22]

DISCUSSION

THE JAPANESE MINISTRY of Health, Labor and Welfare approved interferon, LMV, and ETV as the first-line drugs for HBV infection. ETV has been a safe and effective modality for HBV therapy due to the rarity of resistant HBV strains compared to LMV,[5,6] and 8 years after the initial drug investigation, it is currently regarded as the most favorable medication for HBV infection in Japan.[7] The US Public Health Service's latest guidelines (http://AIDSinfo.nih.gov/contentfiles/AdultandAdolescentGL.pdf), the Japanese guidelines published by the Research Group for Therapy of HIV

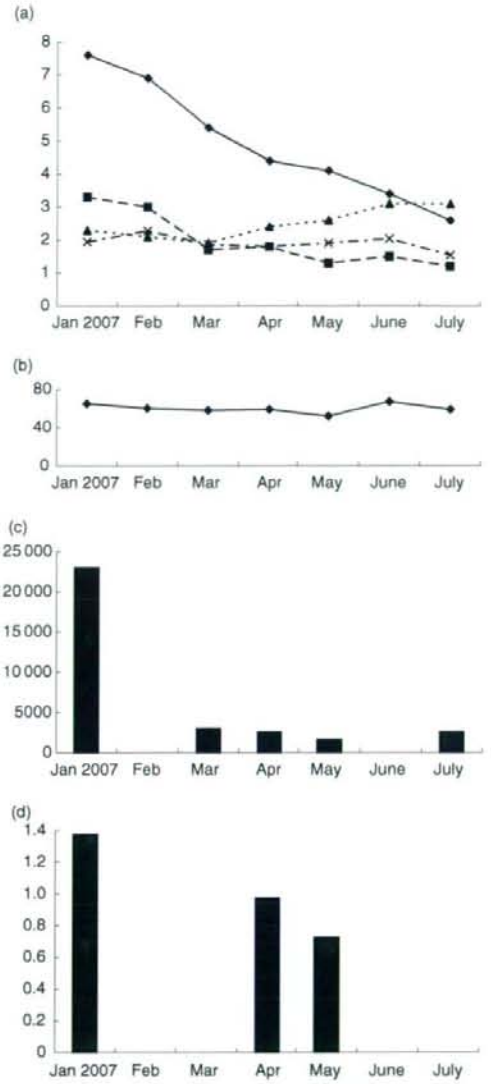


Figure 2 Time-courses of clinical parameters from the initial administration of entecavir on 23 January 2007 demonstrate improvement in liver function and a decrease in hepatitis B virus (HBV) and HIV in the serum. (a,b) HBV load and clinical parameters; (c) HIV load; (d), hepatitis B e antigen (HBe antigen). ALB, albumin; ALT, alanine aminotransferase; INR, international normalized ratio; OD, optical density; PT, prothrombin time; T-bil, total bilirubin. (a) —●— HBV-DNA (log copies/mL), -■- T-BIL (mg/dL), ··▲·· ALB (g/dL), -✱- PT (INR). (b) —●— ALT (IU/l). (c) ■ HIV RNA (copies/mL). (d) ■ HBe antigen (OD at 450 nm).

Infection in December 2006 (http://www.hivjp.org/), and other groups[8] have recommended ETV for hepatitis B treatment in HIV-1-infected people who do not meet the criteria for HIV-1 treatment.[9] The process of establishing the previous consensus should be reviewed. As for the HBV genotype, 85% of HBV-positive patients are genotype C, and 12% of patients are genotype B in Japan.[10] In contrast, only 1–10% of HBV-positive patients carry HBV of genotype A, which is increased mainly in urban districts, probably due to sexual transmission accompanied by chronic outcome.[11,12] The distribution of HBV genotypes in HIV-positive patients in Japan was A (50%), B (5%), C (24%), D (5%), E (2%), H (10%), A plus D (2%), and A plus G (2%).[13] Thus the current case represents the majority of cases of HBV and HIV co-infection in Japan. McMahon *et al.*[14] reported three cases showing an inhibitory effect on HIV by ETV with one case showing selection for the M184V mutation of HIV. They also confirmed that M184V conferred resistance to ETV by *in vitro* experiments. With regard to the current case, one log (10) reduction of HIV RNA load was observed during the 5 months of ETV monotherapy, and HIV mutation relevant to the therapy has been investigated, which will be published. As for HBV, ETV did not select for M204V/I, which is relevant to LMV resistance, and viral reduction of over two log was achieved during 5 month (over 7.6 to under 2.6 log copies/mL) even in the HIV-co-infected case. The genotype A HBV quasispecies pool consisted partly of the HBV −1G precore/core mutant which was revealed to have the higher prevalence in HIV–HBV-co-infected cases compared with the HBV mono-infected case, that is, 20% (5/26) versus 5% (3/62) before LMV therapy, and 40% (20/48) versus 0% (0/62) after LMV therapy.[4] The mutated HBV may need to be supplied with the HBV core protein in trans to form mature HBV particles.[15] An associated high HBV load may hypothetically lead to the progression of liver disease[16] due to the direct cytopathic effect against hepatocytes.[17] The current case suggests evidence of the prevalence of the −1G precore/core deletion mutant that prevails even in Japanese cases. This deletion mutant was originally found in Western countries among HIV–HBV-co-infected cases as a characteristic mutation related to high viral load. The −1G clone diminished during ETV therapy in accordance with the decrease of the viral load of wild strain. The biological and virological significance of the −1G deleted mutant still needs to be clarified. The patient's HBV is currently well managed by ETV and the succeeding combination of FTC and TDF. The numbers of either HBV- or HCV-co-infected HIV cases has risen to 8.8% and 4.3%, respectively, of all HIV-positive cases in Japan.[18] Thus the life-long strategy should be managed based on the latest information. Pegylated interferon, which induces seroconversion of HBe antigen at a higher rate among HBV genotype A-positive patients[19] with preserved liver function, might be an alternative option for ETV. However, interferon therapy was not applied to the current case for its potential of causing acute exacerbation of hepatitis B or underlying autoimmune hepatitis on nearly decompensated livers. Therefore, regardless of the HIV status, patients who need urgent care with severe, impaired liver function may need HAART, including plural anti-HBV nucleoside analogs. Moreover, for co-infected cases, especially for HBV/HIV-co-infected patients with a high HBV load and impaired immune/liver function,[20,21] concomitant or prior therapy against HBV to the anti-HIV therapy is recommended for the risk of flare based on the possible immune reconstitute. HBV and immunological status should be monitored closely in all cases under care in this context.

REFERENCES

1 Lindegren ML, Kobrynski L, Rasmussen SA *et al.* Applying public health strategies to primary immunodeficiency diseases: a potential approach to genetic disorders. *MMWR Recomm Rep* 2004; **53**: 1–29.
2 Soriano V, Miro JM, Garcia-Samaniego J *et al.* Consensus conference on chronic viral hepatitis and HIV infection: updated Spanish recommendations. *J Viral Hepat* 2004; **11**: 2–17.
3 FDA Notifications. Entecavir revision made by FDA and BMS. *AIDS Alert* 2007; **22**: 43.
4 Revill PA, Littlejohn M, Ayres A *et al.* Identification of a novel hepatitis B virus precore/core deletion mutant in HIV/hepatitis B virus co-infected individuals. *Aids* 2007; **21**: 1701–10.
5 Colonno RJ, Rose R, Baldick CJ *et al.* Entecavir resistance is rare in nucleoside naive patients with hepatitis B. *Hepatology* 2006; **44**: 1656–65.
6 Akuta N, Suzuki F, Kobayashi M *et al.* The influence of hepatitis B virus genotype on the development of lamivudine resistance during long-term treatment. *J Hepatol* 2003; **38**: 315–21.
7 Innaimo SF, Seifer M, Bisacchi GS *et al.* Identification of BMS-200475 as a potent and selective inhibitor of hepatitis B virus. *Antimicrob Agents Chemother* 1997; **41**: 1444–8.
8 Benhamou Y. Treatment algorithm for chronic hepatitis B in HIV-infected patients. *J Hepatol* 2006; **44**: S90–4.
9 Rathbun RC, Lockhart SM, Stephens JR. Current HIV treatment guidelines – an overview. *Curr Pharm Des* 2006; **12**: 1045–63.

10 Orito E, Ichida T, Sakugawa H *et al.* Geographic distribution of hepatitis B virus (HBV) genotype in patients with chronic HBV infection in Japan. *Hepatology* 2001; **34**: 590–4.
11 Sugauchi F, Orito E, Ohno T *et al.* Spatial and chronological differences in hepatitis B virus genotypes from patients with acute hepatitis B in Japan. *Hepatol Res* 2006; **36**: 107–14.
12 Ozasa A, Tanaka Y, Orito E *et al.* Influence of genotypes and precore mutations on fulminant or chronic outcome of acute hepatitis B virus infection. *Hepatology* 2006; **44**: 326–34.
13 Shibayama T, Masuda G, Ajisawa A *et al.* Characterization of seven genotypes (A to E, G and H) of hepatitis B virus recovered from Japanese patients infected with human immunodeficiency virus type 1. *J Med Virol* 2005; **76**: 24–32.
14 McMahon MA, Jilek BL, Brennan TP *et al.* The HBV drug entecavir—effects on HIV-1 replication and resistance. *N Engl J Med* 2007; **356**: 2614–21.
15 Gunther S, Piwon N, Jung A *et al.* Enhanced replication contributes to enrichment of hepatitis B virus with a deletion in the core gene. *Virology* 2000; **273**: 286–99.
16 Iloeje UH, Yang HI, Su J *et al.* Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. *Gastroenterology* 2006; **130**: 678–86.
17 Marschenz S, Endres AS, Brinckmann A *et al.* Functional analysis of complex hepatitis B virus variants associated with development of liver cirrhosis. *Gastroenterology* 2006; **131**: 765–80.
18 Gatanaga H, Ibe S, Matsuda M *et al.* Drug-resistant HIV-1 prevalence in patients newly diagnosed with HIV/AIDS in Japan. *Antiviral Res* 2007; **75**: 75–82.
19 Janssen HL, van Zonneveld M, Senturk H *et al.* Pegylated interferon alfa-2b alone or in combination with lamivudine for HBeAg-positive chronic hepatitis B: a randomised trial. *Lancet* 2005; **365**: 123–9.
20 Shire NJ, Sherman KE. Management of HBV/HIV-coinfected patients. *Semin Liver Dis* 2005; **25** (Suppl. 1): 48–57.
21 Drake A, Mijch A, Sasadeusz J. Immune reconstitution hepatitis in HIV and hepatitis B coinfection, despite lamivudine therapy as part of HAART. *Clin Infect Dis* 2004; **39**: 129–32.
22 Takahashi M, Nishizawa T, Gotanda Y *et al.* High prevalence of antibodies to hepatitis A and E viruses and viremia of hepatitis B, C, and D viruses among apparently healthy populations in Mongolia. *Clin Diagn Lab Immunol* 2004; **11**: 392–8.

免疫抑制・化学療法により発症するB型肝炎対策
—厚生労働省「難治性の肝・胆道疾患に関する調査研究」班劇症肝炎分科会および「肝硬変を含めたウイルス性肝疾患の治療の標準化に関する研究」班合同報告—

坪内　博仁　熊田　博光　清澤　研道　持田　智　坂井田　功
田中　榮司　市田　隆文　溝上　雅史　鈴木　一幸　奥芝　眞彰
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