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APPENDIX I

Participating hospitals and clinics

In addition to the hospitals of the study authors, data were supplied by the following hospitals and clinics in the Kyushu Division of the Japanese Society of Gastroenterology: Nippon Steel Yawata Memorial Hospital; Yame General Hospital; 1st Department of Internal Medicine, Ryukyu University School of Medicine; 2nd Department of Internal Medicine, Kagoshima University School of Medicine; Hayato Town Medical Association Medical Center; Department of Internal Medicine, Saga University School of Medicine; Department of Medicine and Biosystemic Science, Kyushu University School of Medicine; Nishinihon Hospital; Kagoshima Kouseiren Hospital; Miyata Memorial Hospital; 2nd Department of Internal Medicine, Nagasaki University School of Medicine; Yonabaru Central Hospital; 2nd Department of Internal Medicine, Miyazaki University School of Medicine; Fukuoka Red Cross Hospital; Kita-kyushu Medical Center Hospital; Munakata Medical Association Hospital; Naika Yamaguchi Urban Clinic; Yamaga Municipal General Hospital; Shin Kokura Hospital; Iizuka Hospital; Oita Prefectural Hospital; 3rd Department of Internal Medicine, University of Occupational and Environmental Health School of Medicine; Kokura National Hospital; Kumamoto Regional Medical Center; Shin Beppu Hospital; Oita National Hospital; Kami Goto Hospital; 1st Department of Internal Medicine, Oita University School of Medicine; National Kyushu Medical Center; 1st Department of Internal Medicine, Nagasaki University School of Medicine; Hirahara Naika Clinic; Kumamoto National Hospital; Saiseikai Fukuoka General Hospital; Arita Ichou Hospital; Labour Welfare Corporation Moji Rosai Hospital; 1st Department of Internal Medicine, Kumamoto University; Kumamoto Red Cross Hospital; National Kyushu Cancer Center Hospital; Kagoshima Kyousaikai Nanpuh Hospital; Iwao Hospital; Saga Social Insurance Hospital; Labour Welfare Corporation Nagasaki Rosai Hospital; Oita Red Cross Hospital; Koebaru Central Hospital; National Sanatorium Kumamoto South Hospital; 2nd Department of Internal Medicine, Oita Medical University; Labour Welfare Corporation Kumamoto Rosai Hospital; Amakusa Regional Medical Center; Miyuki Hospital; Saiseikai Futsukaichi Hospital; Hakujyuji Hospital; Hakuaikai Hospital.

肝臓　47巻7号　355-356（2006）

＜短　報＞

C型慢性肝炎に対するPEG-IFNα2b＋ribavirin併用療法における早期治療効果予測
—血中HCV抗原によるモニタリング解析—

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緒言：難治性C型慢性肝炎の治療は，Pegylated IFN（Peg IFN）とRibavirin（RBV）併用療法が標準的治療法になってきているが，ウイルス学的著効（Sustained virological response：SVR）率は50％に過ぎない[1)2)]．

近年，SVR予測因子として治療直後から治療開始後4週間以内（第1相から第3相）のウイルス減少，治療開始後12週目・24週目のHCVRNA陰性化などウイルス動態が重要となってきている[3)~5)]．今回，我々は難治性C型慢性肝炎に対してPeg IFNとRBV併用療法（併用療法）を行い，治療経過中のHCV Core抗原（HCV抗原）を測定し，治療早期に効果予測が可能であるかを検討した．

対象と方法：当院において2005年1月から5月までに併用療法を行った症例のうち，書面で同意を得たC型慢性肝炎患者45例（Genotype 1b，アンプリコアHCVモニターv2.0オリジナル法：Amplicor-Mで100KIU/ml以上）を対象とした．併用療法12週目まで投与を行った症例の治療前，治療中3日目，1，2，4，12週目の凍結保存（−80℃）血清検体を解析に用いた．併用療法は，体重換算を行い，PEG-IFNα-2b80～120μg/回・皮下注・週1回・48週間投与にRibavirin600～1000mg/日・経口・連日48週間投与を併用した．併用療法の治療効果は，治療12週目のHCVRNA定性陰性もしくはHCV抗原量2Log以上の減少例をEarly Virological Response（EVR），それ以外をNon Early Virological Response（NEVR）とした．両群間の有意差検定は，Mann-Whitney's U-testおよびFisher's exact testを用いて行った．HCV抗原量の測定（オーソHCV抗原IRMAテスト，オーソクリニカル・ダイアグノスティックス）は，血清中のHCVウイルス粒子からエンベロープを除去し，Core粒子を構成単位のCore抗原に分解する．同時に共存するHCVCore蛋白に対する抗体を失活させる．この処理済み検体をHCVコア領域に特異的なモノクローナル抗体を用いた2ステップサンドイッチ法にて補足する．これに^{125}I標識抗ペルオキシダーゼポリクローナル抗体を反応させ，放射線量（cpm）を計測する．HCV抗原の測定値は，7濃度のスタンダードのcpm値より作成した検量線よりHCV抗原量（fmol/L）を算出した．HCV抗原の測定結果は，20fmol/L以下をHCV抗原陰性，20fmol/L以上HCV抗原陽性と判定した．

成績：45例中EVRは36例（80％），NEVRは9例（20％）であった．EVR群の平均年齢は55.8±3.2歳，男女比は22：14，治療前Peg-IFN量92.5±14.1，治療前RBV量731±131，治療前HCV量は抗原で5387±4800fmol/L，Amplicor-Mで383±158KIU/mLであった．一方，NEVR群の平均年齢は57.2±6.7歳，男女比は4：5，治療前Peg-IFN量88.9±10.5，治療前RBV量689±105，治療前HCV量は抗原で4577±2064fmol/L，Amplicor-Mで456±86KIU/mLであった．両群間に有意差は認めなかった．

併用療法での治療効果別，HCV抗原量の推移をFig. 1に示す．HCV抗原量は治療直後より3日目にかけて急激に減少し，3日目より1週目にかけて反跳上昇した．この時点において治療前HCV抗原量を100％として1週目HCV抗原量の50％減少率を検討するとEVR群では100％（36/36），NEVR群では33％（3/9）でNEVR群に比し，EVR群で有意にHCV抗原量の減少を示した（$p<0.01$）．さらに1週目から12週目にかけてもNEVR群に比し，EVR群の方が明らかにHCV抗原量低値で推移した（$p<0.01$）．治療開始12週目の効果予測の可能性をHCV抗原量治療2週目1000fmol/Lを基準値として検討した．HCV抗原量治療2週目1000fmol/L以上は，8例でうち6例（75％）が治療12週目HCV抗原陽性を示した．一方，HCV抗原量治療2週目1000fmol/L未満は，37例でうち35例（95％）が治療12週目HCV抗原陰性を示した．

考察：わが国でも医療保険の適用になって以来，PEG-IFN＋Ribavirin併用療法が盛んに施行されている．しかし，一方で副作用・費用対効果に対しても十分考慮し慎重に治療しなければいけない．この点からも治療開始早期における効果予測が重要となってくる．最近，HCV動態の解析が海外，国内において報告されている[3)~7)]．今回の検討では，最終治療効果判定までには至っていないも，治療開始12週目時点までの効果予測が治療早期HCV抗原量を測定することにより可能であった．とくに1週目のHCV抗原50％減少率で

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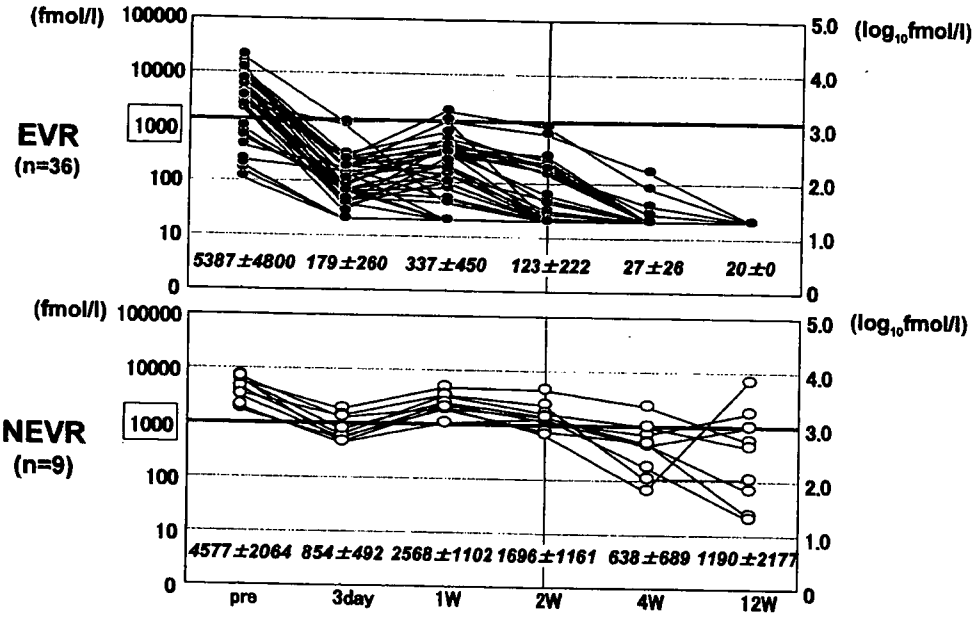

Fig. 1 Serum levels of HCV core antigen during combination therapy of PEG-IFNα2b and ribavirin

はEVR群とNEVR群間において減少量に有意な差が認められた．また，2週目のHCV抗原量からは基準値（1000fmol/L）を設定することにより12週目の効果予測が可能であった．このことから安価で簡便なHCV抗原測定法は，HCVRNA測定法と同様に早期治療効果予測に有用であることが示唆された．

索引用語：HCV抗原，HCV動態

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英文要旨

An early prognosis for the treatment of Chronic Hepatitis C patients with Pegylated Interferon-α2b plus Ribavirin
—Monitoring analysis by the serum HCV core antigen values—

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The capability of an early prognosis for the treatment of Chronic Hepatitis C (CH-C) patients with genotype 1b and high viral loads with Pegylated Interferon-α2b plus Ribavirin was evaluated by the levels of serum HCV core antigens. Serum samples were drawn before the treatment, 3 days or 1, 2, 4, and 12 weeks after the treatment. Out of 45 cases with CH-C, 36 cases (80%) and 9 cases (20%) were early virological response (EVR) and non-early virological response (NEVR), respectvery.

The serum levels of core antigen in EVR were significantly lower than those in NEVR from the third day to the 12th week after the treatment. The levels of core antigen less than 1000fmol/L in the second week could predict a better prognosis in the 12th week. The measurement of serum HCV core antigen was useful for the early prognosis of the treatment in CH-C patients with genotype 1b and high viral loads with Pegylated Interferon-α2b plus Ribavirin.

Kanzo 2006; 47: 355—356

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MAJOR ARTICLE

T1653 Mutation in the Box α Increases the Risk of Hepatocellular Carcinoma in Patients with Chronic Hepatitis B Virus Genotype C Infection

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Background. Most patients with chronic hepatitis B virus infection become carriers of inactive virus after hepatitis B e antigen seroconversion; however, a subgroup of patients have persistent abnormal transaminase levels and develop hepatocellular carcinoma after seroconversion.

Methods. In an age-matched case-control study, 40 carriers of inactive virus (mean age ± standard deviation [SD], 50.9 ± 11.1 years), 40 patients with chronic hepatitis (mean age ± SD, 50.2 ± 8.9 years), and 40 patients with hepatocellular carcinoma (mean age ± SD, 50.7 ± 9.4 years) who were infected with hepatitis B virus genotype C and had test results positive for antibody to hepatitis B e antigen were analyzed.

Results. The prevalence of T1653 in the box α was significantly higher among patients with hepatocellular carcinoma than among carriers of inactive virus who did not have hepatocellular carcinoma (70% vs. 25%; $P < .0001$) or chronic hepatitis (70% vs. 35%; $P = .003$). Mutations in the basic core promoter region (T1762/A1764) were frequently found in all groups, regardless of clinical status (in 77.5% of carriers of inactive virus, 77.5% of patients with chronic hepatitis, and 90% of patients with hepatocellular carcinoma). In the multivariate analysis, the presence of T1653, an alanine aminotransferase level of ≥37 U/L, and a platelet count of $<18 \times 10^4$ platelets/mm^3 were independent predictive values for hepatocellular carcinoma (odds ratio [95% confidence interval], 5.05 [1.56–16.35], 12.56 [3.05–51.77], and 11.5 [3.47–38.21], respectively). High α-fetoprotein level was the only independent predictive value for T1653 in patients with hepatocellular carcinoma (odds ratio, 12.67; 95% confidence interval, 1.19–134.17]). Among patients with test results positive for antibody to hepatitis B e antigen who had hepatocellular carcinoma and were infected with different genotypes of hepatitis B virus, the prevalence of T1653 was 40%, 15%, 25%, 25%, 67%, and 23% in patients infected with hepatitis B virus genotypes Aa, Ae, Ba, Bj, C, and D, respectively ($P < .05$ for genotype C vs. genotypes Ae, Ba, Bj, or D).

Conclusions. Our data indicate that the addition of T1653 mutation in the box α to the basic core promoter mutation increases the risk of hepatocellular carcinoma in patients with hepatitis B virus genotype C.

Hepatocellular carcinoma (HCC) is the fifth most frequent cancer and the third leading cause of cancer-related death in the world, with an estimated prevalence of >500,000 cases worldwide per year [1]. It is now accepted that hepatitis B virus (HBV) has a carcinogenic potential in humans. Several mutations in the HBV genome have been reported to occur during the course of persistent viral infection, and there has been increasing evidence of an association between these molecular alterations and the development of HCC in patients with HBV infection.

During persistent HBV infection, carriers frequently undergo seroconversion from hepatitis B e antigen (HBeAg) to the corresponding antibody (anti-HBe). Most patients who acquire chronic HBV infection with HBV genotype C (which is a common genotype in East

Received 17 July 2005; accepted 23 August 2005; electronically published 29 November 2005.

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Clinical Infectious Diseases 2006; 42:1–7

1058-4838/2006/4201-0001$15.00

Asian countries) by perinatal transmission become carriers of inactive virus after seroconversion. A subgroup of patients have persistent abnormal serum transaminase levels and develop HCC in the anti-HBe–positive phase. Many of these patients have active viral replication and are infected with several mutant viruses. The association between different clinical events after seroconversion and specific HBV genomic mutations has not been clearly defined.

Mutations in the basic core promoter (BCP) region at nucleotides (nt) 1762/1764 (T1762/A1764) and mutation in the precore (preC) region at nt 1896 (A1896) are associated with seroconversion and persistent viral replication. It is noteworthy that both BCP and preC mutations are often found in patients with advanced liver disease, (e.g., HCC) [2–8]. The T1762/A1764 mutation alters HBeAg production at the transcription level, and the A1896 in the preC region terminates translation of the precursor protein, abrogates HBeAg production, and results in seroconversion. A1896 was also reported previously to be associated with severe forms of chronic liver disease [7,8].

HBV has been classified into 8 major genotypes with use of the complete nucleotide sequence of the viral genome [10]. HBV genotypes not only have distinct geographical distributions [7, 11, 12] but also have different clinical manifestations and responses to therapy (e.g., IFN therapy). Furthermore, HBeAg positivity and levels of HBV DNA, which are controlled by specific mutations, differ between HBV genotypes (e.g., the BCP double mutation is more prevalent among strains of HBV genotype C, followed by HBV genotype A, and the A1896 mutation is frequently found in HBV genotypes B and D) [13–16].

There have been many studies involving viral mutations associated with clinical features, but most previous studies have ignored age, sex, HBeAg status, and HBV genotypes. In Japan, most patients with HCC experience seroconversion (i.e., they are anti-HBe positive) and have HBV genotype C; therefore, we performed an age-matched case-control study among anti-HBe–positive patients infected with HBV genotype C (including carriers of inactive virus, patients with chronic hepatitis, and patients with HCC) to determine the specific HBV genome mutations associated with disease progression.

PATIENTS AND METHODS

Serum samples. Serum samples were obtained from 211 patients from different regional areas worldwide. A total of 120 patients from Japan who were infected with HBV genotype C (40 carriers of inactive virus, 40 patients with chronic hepatitis, and 40 patients with HCC) were matched with control subjects according to age and HBe status. Control serum samples were obtained from patients with HCC who were positive for anti-HBe and who were infected with HBV genotype Aa (10 subjects), Ae (13), Ba (20), Bj (20), C (15), and D (13). Control subjects were from Hong Kong (19 subjects), Japan (36), and the United States (36). The majority of patients infected with HBV genotypes Aa, Ba, Bj, and C were Asian, and the majority of patients infected with HBV genotypes Ae and D were white and black. None of the subjects had serological test results positive for markers of infection with hepatitis C virus or HIV-1.

The study protocol was approved by ethics committees of the participating institutions in accordance with the 1975 Helsinki declaration. Informed consent was obtained from each patient.

Serological assays for HBV markers. HBeAg and anti-HBe were detected by chemiluminescent EIA (Lumipulse *f*, Fujirebio). HBV genotypes were determined by the restriction fragment–length polymorphism method on the S gene sequence amplified by PCR [29] and ELISA with monoclonal antibodies directed to distinct epitopes on the preS2 region products [18], with use of commercial kits (HBV genotype EIA; Institute of Immunology). The genotypes were also confirmed with use of a phylogenetic tree analysis. α-Fetoprotein and serum protein induced by the absence of vitamin K (antagonist II) were examined with use of chemiluminescent EIA.

Amplification and sequencing of the core promoter and the precore region plus core gene. HBV DNA sequences bearing the core promoter and preC or core regions were amplified by PCR with heminested primers by the method described elsewhere [19]. Thereafter, PCR products were sequenced directly with Prism Big Dye (Applied Biosystems) in the ABI 3100 DNA automated sequencer (Applied Biosystems). Accession numbers for all strains are AB236515–AB236634.

Case-control study. A carrier of inactive virus was defined as an HBsAg-positive individual with normal alanine aminotransferase (ALT) levels for a 2-year period (with at least 4 evaluations at 3-month intervals) and without the presence of portal hypertension. Chronic hepatitis was defined as persistent elevation of ALT levels (> 1.5 × upper limit of normal [35 U/L]) during a 6-month period (with at least 3 evaluations at 2-month intervals) without a decrease in platelet count or albumin level, and hypersplenism (splenomegaly on ultrasonographic examination). Twenty-one patients were confirmed to have chronic hepatitis by means of a fine-needle biopsy of the liver. Staging and grading (expressed as mean value ± SD [95% CI]) were 1.24 ± 0.64 (0.99–1.58) and 1.36 ± 0.58 (1.07–1.59), respectively, as previously described [30]. None had received antiviral treatment during the follow-up period. Of 40 patients with HCC, 23 patients received a diagnosis of HCC on the basis of a pathologic examination, and 17 patients received a diagnosis of HCC on the basis of results of abdominal ultrasonography, angiography, CT, or MRI, as well as an elevated serum α-fetoprotein level (≥400 ng/mL).

Statistical evaluation. Data were expressed as mean ±

SD. Statistical analyses were performed using χ^2 test and Fisher's exact test for categorical variables. Mann-Whitney *U* test or 1-way analysis of variance were used for continuous variables, as appropriate. Mantel-Haenszel χ^2 test was used to analyze the trend of frequencies of viral mutations. Multivariate analyses with logistic regression were used to determine the independent factors associated with HCC and T1653. Differences were considered to be significant for *P* values <.05. The statistical analysis software used was Stata software, version 8.0 (StataCorp).

RESULTS

Table 1 compares ALT level, platelet count, and HBV DNA level, as well as mutations in the box α (enhancer II), core promoter, and preC region, among 40 carriers of inactive virus, 40 patients with chronic hepatitis, and 40 patients with HCC who were infected with HBV genotype C in an age-matched case-control study. ALT and HBV DNA levels were significantly lower among carriers of inactive virus than among patients with chronic hepatitis or patients with HCC ($P < .0001$ and $P = .001$, respectively). Platelet count was lower among patients with HCC than among carriers of inactive virus or patients with chronic hepatitis ($P < .0001$).

The frequency of the T1653 mutation in the box α was significantly higher among patients with HCC (70%) than among carriers of inactive virus (25%) or patients with chronic hepatitis (35%; $P < .0001$) (table 1). Of interest, the T1653 mutation had an opposite correlation with the M1753 mutation. The prevalence of T1762/A1764 was high in all clinical status groups, with no statistically significant difference between groups (table 1). The trend of the frequency of T1653, increasing from carriers of inactive virus to patients with chronic hepatitis to patients with HCC, was analyzed by Mantel-Haenszel χ^2 test (OR, 2.48; 95% CI, 1.59–3.85; $P = .0001$) (figure 1). The trend of the frequency of T1762/A1764 was not statistically significant ($P = .1502$) (figure 1).

The attributable risk of multiple factors, including sex, HBV DNA level, ALT level, platelet count, and the presence of the T1653, M1753, T1762/A1764, and A1896 mutations for HCC in the HBV carriers was determined by multiple logistic regression analysis (table 2). There was a statistically significant association between development of HCC and ALT level >37 U/L (OR, 12.56; 95% CI, 0.55–6.21; $P < .0001$) and platelet count $<18 \times 10^4$ platelets/mm^3 (OR, 11.5; 95% CI, 3.47–38.21; $P < .0001$). The T1653 mutation was still significantly associated with the development of HCC (OR, 5.05; 95% CI, 1.56–16.35; $P = .007$).

The attributable risk of multiple factors, including HBV DNA level, ALT level, platelet count, α-fetoprotein level, protein in-

Table 1. Demographic, clinical, and virologic characteristics of patients infected with hepatitis B virus (HBV) genotype C who were matched for age and hepatitis B e antigen (HBeAg) status.

Variable	Clinical status			
	Carriage of inactive virus (*n* = 40)	Chronic hepatitis (*n* = 40)	Hepatocellular carcinoma (*n* = 40)	*P*
Male sex	31 (77.5)	37 (92.5)	36 (90)	.171
Age, years	50.9 ± 11.1	50.2 ± 8.9	50.7 ± 9.4	Matched
HBeAg positive	0 (0)	0 (0)	0 (0)	Matched
Anti-HBeAg positive	40 (100)	40 (100)	40 (100)	Matched
HBV genotype C	40 (100)	40 (100)	40 (100)	Matched
Alanine transaminase level, U/L[a]	20.8 ± 7.6	102 ± 108.7	83.2 ± 84.8	.0001
Platelet count, $\times 10^4$ platelets/mm^3[b]	20.7 ± 3.1	17.4 ± 4.1	12.8 ± 5.7	.0001
HBV DNA level, LGE/mL[c]	4.3 ± 0.8	5.9 ± 1.5	5.4 ± 1.5	<.0001
Mutation in the box α: T1653[d]	10 (25)	14 (35)	28 (70)	<.0001
Mutation in the core promoter				
M1753	10 (25)	6 (15)	9 (22.5)	.609
T1762/A1764	31 (77.5)	31 (77.5)	36 (90)	.289
Mutation in the precore region: A1896	25 (62.5)	26 (65)	25 (62.5)	1.0

NOTE. Data are no. (%) of patients or mean value ± SD. Anti-HBeAg, antibody to HBeAg; LGE, log genome equivalents.

[a] $P < .0001$ for carriers of inactive virus vs. patients with chronic hepatitis; $P = .002$ for carriers of inactive virus vs. patients with hepatocellular carcinoma.

[b] $P < .0001$ for patients with hepatocellular carcinoma vs. carriers of inactive virus or patients with chronic hepatitis; $P = .002$ for carriers of inactive virus vs. patients with chronic hepatitis.

[c] $P < .0001$ for carriers of inactive virus vs. patients with chronic hepatitis; $P = .001$ for carriers of inactive virus vs. patients with hepatocellular carcinoma.

[d] $P < .0001$ for carriers of inactive virus vs. patients with chronic hepatitis; $P = .001$ for carriers of inactive virus vs. patients with hepatocellular carcinoma.

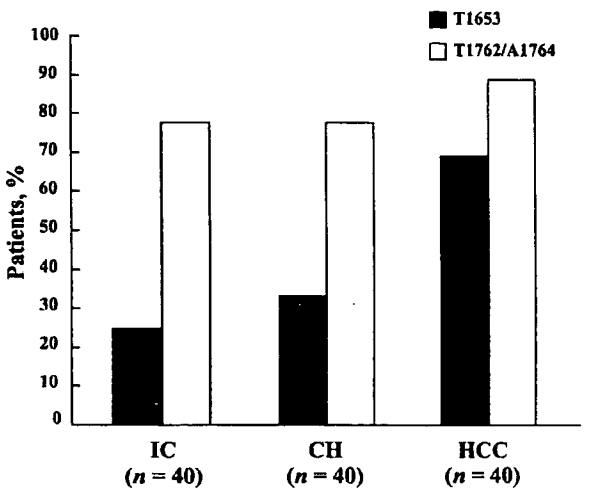

Figure 1. Prevalence of T1653 box α and T1762/A1764 basic core promoter mutations among patients with chronic hepatitis B virus infection, stratified by clinical status. The trend of the frequency of the T1653 mutation was analyzed by Mantel-Haenszel χ^2 test. The OR estimate is an approximation of the OR for carriers of inactive virus (IC), patients with chronic hepatitis (CH), and patients with hepatocellular carcinoma (HCC) having a strain with the mutation (OR, 2.48; 95% CI, 1.59–3.85; $P = .0001$). The trend of the frequency of the T1762/A1764 mutation was not statistically significant according to the Mantel-Haenszel χ^2 test ($P = .1502$).

duced by the absence of vitamin K (antagonist II) level, for T1653 in patients with HCC with HBV genotype C infection was determined by multiple logistic regression analysis (table 3). An α-fetoprotein level >300 ng/mL was the only independent predictive value for the presence of the T1653 mutation in patients with HCC with HBV genotype C infection (OR, 12.67; 95% CI, 1.19–134.17; $P = .035$).

Table 4 compares sex, age, and mutations in the box α, core promoter, and preC region among patients infected with HBV genotypes Aa (10 patients), Ae (13), Ba (20), Bj (20), C (15), and D (13) with the same variables among patients with HCC. Mean age was significantly higher among patients with HBV genotype Bj infection, compared with patients with HBV genotype Ba, genotype C, and genotype D infection ($P < .05$). The prevalence of T1653 among patients with HBV genotype C infection (66.7%) was significantly higher than it was among patients infected with other genotypes (15%–25%; $P < .05$), excluding patients infected with HBV genotype Aa. The prevalence of T1762/A1764 among patients with HBV genotype Ba infection (85%) and HBV genotype C infection (86.7%) was also significantly higher than it was among patients infected with other genotypes (20%–50%; $P < .05$). The prevalence of A1896 among patients with HBV genotype Aa infection and HBV genotype Ae infection was significantly lower than it was among patients infected with other genotypes ($P < .05$).

DISCUSSION

Many previous studies have reported that the clinical course of chronic HBV infection may be modified by several specific viral mutations [5, 20, 21], although the significance of such specific mutations in patients with chronic hepatitis B remains controversial. Because most studies have not controlled for different variables, such as age, HBV genotype, and HBe status, it is unknown whether the mutations were associated with disease progression, greater age of the patient, the specific HBV genotype or subtype, or HBe status. In this study, to exclude any biases, we performed an age-matched case-control study involving only anti-HBe–positive patients infected with HBV genotype C.

In the present case-control study, the prevalence of T1653 was found to be significantly higher among patients with HCC, compared with carriers of inactive virus and patients with chronic hepatitis with HBV genotype C infection; however, the prevalence of T1762/A1764 was high in all clinical status groups. During the anti-HBe–positive phase of infection, T1653 was more reliable than T1762/A1764 as a predicting factor for

Table 2. Multivariate analysis of variables with independent predictive value for development of hepatocellular carcinoma among a group of 120 patients with hepatitis B virus infection.

Variable	OR (95% CI)	P
Sex		
Female	1	
Male	5.06 (0.85–30.15)	.075
HBV DNA level		
<4.8 LGE/mL	1	
≥4.8 LGE/mL	0.34 (0.09–1.21)	.096
Alanine transaminase level		
<37 U/L	1	
≥37 U/L	12.56 (3.05–51.77)	.0001[a]
Platelet count		
≥18 × 10^4 platelets/mm^3	1	
<18 × 10^4 platelets/mm^3	11.51 (3.47–38.21)	.0001[a]
T1653 mutation		
No	1	
Yes	5.05 (1.56–16.35)	.007[a]
M1753 mutation		
No	1	
Yes	1.23 (0.31–5.04)	.770
T1762/A1764 mutation		
No	1	
Yes	2.67 (0.57–12.54)	.214
A1896 mutation		
No	1	
Yes	0.96 (0.29–3.11)	.943

NOTE. Each OR was adjusted for age and other variables in the analysis. LGE, log genome equivalents.

[a] Statistically significant.

Table 3. Multivariate analysis of variables with independent predictive value for the presence of the T1653 mutation among 40 patients with hepatocellular carcinoma.

Variable	OR (95% CI)	P
HBV DNA level		
<4.9 LGE/mL	1	
≥4.9 LGE/mL	0.89 (0.16–4.79)	.899
ALT level		
<53 U/L	1	
≥53 U/L	1.72 (0.29–9.96)	.541
Platelet count		
≥12 × 10^4 platelets/mm^3	1	
<12 × 10^4 platelets/mm^3	1.39 (0.28–7.02)	.683
α-Fetoprotein level		
<300 ng/mL	1	
≥300 ng/mL	12.67 (1.19–134.17)	.035[a]
PIVKA-2 level		
<50 mAU/mL	1	
≥50 mAU/mL	0.25 (0.05–1.43)	.120

NOTE. Each OR was adjusted for age and other variables in the table. PIVKA-2, protein induced by the absence of vitamin K (antagonist II).

[a] Statistically significant.

the development of HCC. In fact, in the multivariate analysis, the presence of T1762/A1764 was not an independent predictor of HCC, but ALT level >37 U/L, platelet count <18 × 10^4 platelets/mm^3, and the presence of T1653 were independent predictors of HCC. The T1653 mutation had also been reported by Takahashi et al. [17]; they reported that this specific mutation was prevalent among Japanese patients with HCC, although their study was not a case-control study. These results do not deny that T1762/A1764 is associated with hepatocarcinogenesis, because poor prognosis associated with HBV genotype C infection, compared to that associated with HBV genotype B (Ba and Bj) infection, correlated with a high prevalence of T1762/A1764 [2, 9, 16], indicating that the BCP double mutation is associated with a high potential for hepatocarcinogenesis. The appearance of the T1653 mutation after the occurrence of the T1762/A1764 mutation (the T1762/A1764 mutation usually occurs earlier than the T1653 mutation) could indicate that the virulence of HBV is increasing, which could result in the development of HCC. In the multivariate analysis, however, HBV DNA level was no longer a predicting factor for HCC. One of the reasons for this is that the HBV DNA data used in this study were obtained at the time of diagnosis of HCC. A recent prospective study from Taiwan has indicated that high HBV DNA levels at baseline and infection with HBC genotype C were independent predictors for HCC, but the mean viral load at the time of diagnosis of HCC was significantly lower than at baseline [27]. Although our data could not indicate an association between HBV DNA level and hepatocarcinogenesis, if we could measure the HBV DNA level before diagnosis of HCC, it might found to be be a predicting factor for HCC. Furthermore, an examination of the characteristics of patients with HCC who had the T1653 mutation showed that an elevated α-fetoprotein level (≥300 ng/mL) was the only predictor for the development of HCC in patients with the T1653 mutation. It has been reported that α-fetoprotein level is useful not only for diagnosis but also as a prognostic indicator for patients with HCC [22, 23], suggesting that the T1653 mutation might be associated with poor prognosis for patients with HCC.

The prevalence of several mutations among patients with HCC differed from that among patients with different HBV genotypes (Aa, Ae, Ba, Bj, C, and D) (table 4). The prevalence

Table 4. Demographic and virological characteristics of patients with hepatocellular carcinoma who were positive for antibody to hepatitis B e antigen (anti-HBe), by hepatitis B virus (HBV) genotype.

	HBV genotype						
Variable	Aa (n = 10)	Ae (n = 13)	Ba (n = 20)	Bj (n = 20)	C (n = 15)	D (n = 13)	P
Male	10 (100)	12 (92.3)	18 (90)	15 (75)	15 (100)	13 (100)	.10
Age, years[a]	54.4 ± 7.7	55.3 ± 4.4	54.4 ± 14.8	64.9 ± 9.6	47.9 ± 7.6	53.5 ± 8.3	.0002
HBeAg positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	Matched
Anti-HBe positive	10 (100)	13 (100)	20 (100)	20 (100)	15 (100)	13 (100)	Matched
Mutation in the box α: T1653[b]	4 (40)	2 (15.4)	5 (25)	5 (25)	10 (66.7)	3 (23.1)	.039
Mutations in the core promoter region							
M1753	3 (30)	3 (23.1)	5 (25)	4 (20)	2 (13.3)	1 (7.7)	.759
T1762/A1764[c]	5 (50)	6 (46.2)	17 (85)	4 (20)	13 (86.7)	5 (38.5)	<.0001
Mutation in the precore region: A1896[d]	0 (0)	0/13 (0)	9/20 (45)	15/20 (75)	9/15 (60)	8/13 (61.5)	<.0001

NOTE. Data are no. (%) of patients or mean value ± SD. HBeAg, hepatitis B e antigen.

[a] $P < .05$ for Bj vs. Ba or D; $P < .0001$ for Bj vs. C.

[b] $P < .05$ for C vs. Ba or Bj or D; $P < .01$ for Ae vs. C.

[c] $P < .05$ for Ae vs. Ba or C; $P < .01$ for D vs. Ba or C; $P < .0001$ for Bj vs Ba or C.

[d] $P < .05$ for Ba vs. Aa or Ae; $P < .005$ for Aa vs. C or D and for Ae vs. Ba or C or D; $P < .0001$ for Bj vs Aa or Ae.

of T1653 was the highest among patients with HBV genotype C infection, followed by those with HBV genotype Aa infection, although the number of patient with HBV genotype Aa infection was too small for any conclusions to be drawn. The prevalence of T1762/A1764 was higher among patients with HBV genotype Ba and HBV genotype C infection than among patients infected with other genotypes. HBV genotype Ba has a sequence that closely resembles that of HBV genotype C in the core promoter region, because it is recombinant HBV between HBV genotype Bj and HBV genotype C from nucleotides 1740 to 2485. Although A1896 was not found in HBV genotype Aa and HBV genotype Ae, as has been reported elsewhere [15], HBV genotype Aa had some specific mutations upstream of the preC initiation codon and encapsidation signal site. Therefore, several HBV genotype–specific mutations would be associated with different mechanisms on seroconversion or HBV replication for each genotype or subtype.

Buckwold et al. [24] reported that T1762/A1764 can no longer bind liver-enriched transcription factors and that the transcription of precore RNA and the expression of HBeAg were reduced. Thereafter, Li et al. [25] reported that this mutation not only removed the nuclear receptor–binding site but also created a hepatic nuclear factor 1 transcription factor–binding site. As for a factor correlated with BCP, the core upstream regulatory sequence, which has a strong stimulation effect on the BCP, was reported. In an earlier article by Yu et al. [28], the box α elements (nucleotides 1646–1668) individually stimulated promoter activity >100-fold. The T1653 mutation converts the box α binding site for CCAAT/enhancer-binding protein and related factors into the perfect palindromic sequence 1648-TCTTATATAAGA, which might enhance binding affinity and core promoter/enhancer II activity. Therefore, it is possible that the mutation in the box α influenced the HBe production and viral replication through the BCP activity. In addition, the T1653 mutation corresponds to an amino acid change from histidine to tyrosine at aa 94 of the X protein, so this alteration of X protein might be hepatocarcionogenesis. Gunther et al. [26] analyzed T1653, T1762, and A1764 mutations in the context of an in vitro study involving wild-type HBV (genotype D, AF043594), and they reported that the preC mRNA and HBeAg secretion was reduced, but the amount of progeny virus DNA in the cells and in the culture medium increased only marginally (if at all), as determined by Southern blot analysis. However, because the genotype was different from that in our study (genotype D vs. genotype C) and the mutant type included not only T1653, T1762, and A1764 mutations but also other mutations in the core promoter, it is possible that some other mutation influenced the results in the earlier study.

In conclusion, the addition of the T1653 mutation in the box α to the BCP mutation increases the risk of HCC in patients with HBV genotype C infection, suggesting that HBV with both the T1653 mutation and the BCP double mutation in patients with chronic hepatitis B should be eradicated by antiviral therapy. Functional analyses of HBV strains with the T1653 mutation are needed in vitro and in vivo.

Acknowledgments

We greatly appreciate Dr. Takaji Wakita (Department of Microbiology, Tokyo Metroporitan Institute of Neuroscience, Tokyo, Japan), for his enlightening advice.

Financial support. The Ministry of Health, Labour, and Welfare of Japan (H16-kanen-3), the Ministry of Education, Culture, Science, and Sports of Japan (grants-in-aid for Young Scientists [A] 16689016), and the Uehara Memorial Foundation.

Potential conflicts of interest. All authors: no conflicts.

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J Gastroenterol 2005; 40:204–208
DOI 10.1007/s00535-004-1525-4

Journal of Gastroenterology

Case report

Juvenile hepatocellular carcinoma with congestive liver cirrhosis

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A case of juvenile hepatocellular carcinoma (HCC) with congestive liver cirrhosis is reported. The patient was a 21-year-old woman. She had been diagnosed as having transposition of the great arteries, type 2, in 1978. She underwent the Mustard operation, but suffered from chronic heart failure. In 1995, she experienced abdominal pain and underwent examination. The laboratory data were normal, except for elevated total bilirubin (5.2 mg/dl). Blood examinations were performed at frequent intervals, and the total bilirubin level fluctuated between 0.9 and 8.1 mg/dl over the next 4 years, but the transaminase level remained normal. In 1999, she experienced abdominal pain again and was admitted to our hospital. Computed tomography showed four space-occupying lesions in the liver; 45 mm, 20 mm, 12 mm, and 10 mm in size. She was diagnosed as having HCC, and transcatheter arterial chemoembolization and percutaneous ethanol injection therapy were performed. Histology of the cancerous and the noncancerous liver tissue revealed HCC, moderately differentiated type, in cirrhotic liver with congestion. This patient had no background factors of liver disease, except for liver congestion, associated with the chronic heart failure. Because most patients with cardiac cirrhosis die of cardiac disease, only a small number of these patients develop liver failure. However, the incidence of HCC in patients with congestive liver disease is likely to increase in the future, as survival time is prolonged with the advances in treatment for chronic heart failure. Therefore, patients with congestive liver disease should be followed, taking into account the possibility of HCC.

Received: March 29, 2004 / Accepted: June 1, 2004
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Key words: congestive liver cirrhosis, cardiac cirrhosis, juvenile hepatocellular carcinoma

Introduction

Congestive liver disease results from gross outflow block to the hepatic vein. It is caused by the Budd-Chiari syndrome (BCS), or by congestive heart failure. There are reports of some patients in whom BCS is complicated by hepatocellular carcinoma (HCC). However, there have been no previous reports of HCC occurring with congestive heart failure. According to the Fourteenth Report (1996–1997) of the Liver Cancer Study Group of Japan, of HCC patients in Japan, the rate of juvenile incidence, under age 40 years, is reported to be as low as 1.1%. The most common background factor of juvenile HCC in Japan is continuous infection with hepatitis B virus (HBV). The positivity rate of hepatitis B surface antigen (HBsAg) in juvenile HCC is 66.7%–91.0%.[1–4] We encountered juvenile HCC caused by congestive liver cirrhosis due to chronic heart failure. The patient had no background factors for liver disease except for liver congestion. We report this case and also discuss the relationship between congestive liver cirrhosis and HCC.

Case report

In 1978, the patient, as a newborn female baby, was admitted to Osaka University Hospital with cyanosis. Examination by cardiovascular angiography led to the diagnosis of transposition of the great arteries type 2. She underwent the Mustard operation in 1979, but

continued to suffer from pulmonary hypertension and right-sided heart failure.

In 1995, she complained of right hypochondrial pain. The laboratory data were normal, except for the total bilirubin level: aspartate aminotransferase (AST), 25 U/l; alanine aminotransferase (ALT), 18 U/l; albumin, 3.5 g/dl; total bilirubin, 5.2 mg/dl; and direct bilirubin, 1.3 mg/dl. Ultrasonography and computed tomography (CT) also showed normal findings. She was followed at our hospital, and regularly underwent blood examinations (average interval, 7.6 days). The results showed that the total bilirubin level was elevated (2.9 mg/dl on average), but the transaminase level was normal (Fig. 1).

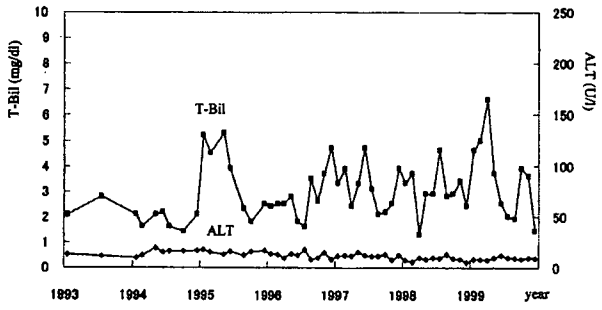

Fig. 1. Blood examination data. The patient was examined at regular intervals from 1995 to 1999. The total bilirubin level was elevated, while the ALT level was normal. *T-Bil*, total bilirubin; *ALT*, alanine aminotransferase

In September 1999, she experienced right hypochondrial pain again and was admitted to our hospital. Ultrasonography revealed a hyperechoic lesion, 50 mm in size, in Couinaud's segment 8 (S8) of the liver.

Physical examination on admission revealed jaundice of the blepharoconjunctival tissue, jugular vein swelling, and cyanosis of the nail bed. Auscultation revealed Levine III/VI degree continuous cardiac murmur in the third intercostal space on the left side of the sternum. Lung fields were clear. She had an enlarged liver that was palpable 4 cm below the right costal margin, with tenderness. There was no neurological abnormality. Laboratory data included the following: AST, 11 U/l; ALT, 8 U/l; albumin, 3.3 g/dl; total bilirubin, 3.9 mg/dl; direct bilirubin, 0.8 mg/dl; α-fetoprotein (AFP), 88 ng/ml; and protein-induced vitamin-K absence-II (PIVKA-II), 704 μg/ml (Table 1). All hepatitis viral markers were negative. She did not habitually consume alcohol or smoke. The findings were negative for both antinuclear antibody and anti-DNA antibody. Antimitochondrial antibody (AMA) was positive, but the AMA titer was low. Because ferric and cuprous metabolic markers were atypical, both hemochromatosis and Wilson's disease were ruled out.

Chest X-ray film showed cardiac dilatation and bulging of the right diaphragm (Fig. 2). The cardiothoracic ratio was 60%. Ultrasonography showed hyperechoic lesions in S8, S7, and S3 of the liver. Unenhanced CT showed space-occupying lesions (SOLs), as low-density areas, in S8, S7/8, and S3 of the liver. These lesions were enhanced in the early phase of incremental CT (Fig. 3abc). In the portal phase, the liver parenchyma showed

Table 1. Laboratory data on admission

Hematology		**Biochemical examination**		**Infection**	
WBC	3840/μl	Na	142 mEq/l	HBsAg	(−) <0.5 U/ml
RBC	540 × 10⁴/μl	K	3.5 mEq/l	Anti-HBs	(−) <10.0 mU/ml
Hb	10.8 g/dl	Cl	110 mEq/l	Anti-HBc IgM	(−) 0.1 CI
Ht	38.4%	BUN	13 mg/dl	Anti-HBc IgG	19.3%
Plt	11.4 × 10⁴/μl	Creatinine	0.5 mg/dl	Dilution (×200)	(−)
		Ca	4.4 mEq/l	HBV-DNA polymerase	0 cpm
Coagulation test		AST	11 U/l	Anti-HAV IgM	(−)
PT-%	40%	ALT	8 U/l	Anti-HCV	(−)
PT-INR	1.80	ALP	184 U/l	RPR	(−)
APTT	43 s	γ-GTP	48 U/l	ATLA	(−)
Fibrinogen	179 mg/dl	LDH	279 U/l	HIV	(−)
FDP	2.95 μg/ml	T-Bilirubin	3.9 mg/dl		
		D-Bilirubin	0.8 mg/dl	**Others**	
Tumor markers		I-Bilirubin	3.1 mg/dl	ANA	(−)
AFP	88 ng/ml	CRP	<0.2 mg/dl	Anti-DNA antibody	(−)
PIVKA-II	704 μg/ml	T-Protein	6.4 g/dl	AMA	×20
CEA	1 ng/ml	Albumin	3.3 g/dl	IgM	104 U/ml
CA19-9	28 U/ml	T-Cholesterol	89 mg/dl	Fe	23 μg/dl
		NH_3	99 μg/dl	Ferritin	10 ng/dl
				Cu	127 μg/dl
				Ceruloplasmin	29 mg/dl

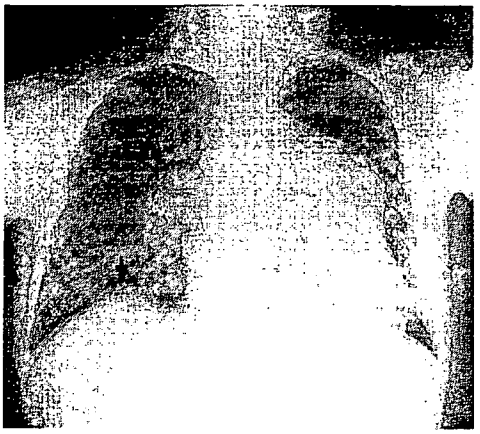

Fig. 2. Chest X-ray. Cardiac dilatation and bulging of the right diaphragm can be seen (*arrow*)

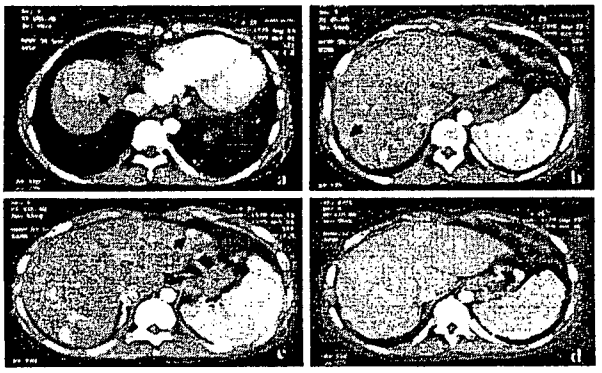

Fig. 3a–d. Abdominal computed tomography (CT) scans. **a** Space-occupying lesion (SOL), 45 mm in size, in S8 of the liver, which was enhanced in the early phase (*arrow*). **b** SOLs, one, 20 mm in size, in S7/8 of the liver, and one, 10 mm in size, in S3 of the liver, which were enhanced in the early phase (*arrows*). **c** SOL, 12 mm in size, in S3 of the liver, which was enhanced in the early phase (*arrow*). **d** In the portal phase, the liver parenchyma was spottily enhanced

spotty enhancement (Fig. 3d). Magnetic resonance imaging (MRI) showed the same SOLs as those described above. Echocardiography revealed hypokinesis and hyposystole of the left ventricle, congestion, and pulmonary hypertension (Fig. 4). Selective angiography of the celiac artery demonstrated hypervascular lesions in S8, S7, and S3 of the liver.

The patient was diagnosed as having HCC, based on these findings. To treat the HCC, transcatheter arterial chemoembolization with emulsion of epirubicin (Farumorubicin; Kyowa Hakko Kogyo, Tokyo, Japan) and Lipiodol (Lipiodol TACE) and percutaneous ethanol injection therapy (PEIT) were performed. After the Lipiodol TACE and PEIT, subsequent examination of AFP and PIVKA-II showed normalized values.

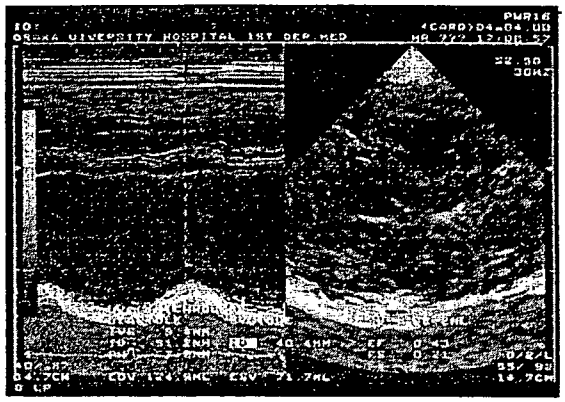

Fig. 4. Echocardiography showed hypokinesis and hyposystole of the left ventricle, congestion, and pulmonary hypertension. LVDd (left ventricular end-diastolic diameter), 53 mm; LVDs (left ventricular end-systolic diameter), 38 mm; *IVST* (interventricular septum thickness), 9.4 mm; *PWT* (posterior wall thickness), 7.8 mm; *EF* (ejection fraction), 43%; *FS* (fractional shortening), 21%; ΔPG (pressure gradient): 38 mm

To investigate the etiology of HCC, needle biopsy of the cancerous and noncancerous liver tissues was performed under ultrasonographic guidance. Histologically, the cancerous liver tissue had large nuclei (Fig. 5a) and was diagnosed as HCC of moderately differentiated type. In the noncancerous liver tissue, fibrous thickening was observed in the region of the central vein (Fig. 5b). The cancerous and the noncancerous liver tissues did not show staining for HBsAg or hepatitis B core antigen (HBcAg). Histological findings suggested that chronic passive congestion was the main cause of the liver cirrhosis.

After discharge from the hospital, the patient was reviewed at regular intervals until November 2000. AFP and PIVKA-II levels remained normal, and further ultrasonography and CT showed no evidence of HCC recurrence during the follow-up period at our hospital.

Discussion

HCC generally arises from chronic hepatitis and cirrhosis. Known etiological factors are hepatitis viral infection; alcoholic liver disease; autoimmune liver diseases, such as autoimmune hepatitis, primary biliary cirrhosis, and primary sclerosing cholangitis; and metabolic liver diseases, such as hemochromatosis and Wilson's disease. Among these etiological factors, the most important is chronic hepatitis viral infection. In a recent

Fig. 5a,b. Histology of the liver. **a** In the cancerous liver tissue, large nuclei are present in the hepatocytes. **b** In the noncancerous liver tissue, fibrous thickening can be observed in the region of the central vein (*CV*). H&E, ×100

report of the Liver Cancer Study Group of Japan, for 1998 to 1999, for HCC patients, the positivity rate for antibody to hepatitis C virus (HCV) was 72.3% and the HBsAg positivity rate was 16.8%.[5] There have been some reports that HCC arose in patients with congestive liver disease, especially in those with the BCS, but there have been no previous reports of HCC occurring with cardiac cirrhosis.

Congestive liver disease is caused by gross outflow block to the hepatic vein. Structural and functional obstruction of the hepatic vein, the inferior vena cava (IVC), and the right atrium impedes hepatic venous drainage, and causes severe chronic venous congestion and centrilobular hypoxia of the liver. With cardiac cirrhosis, hypoxia of the liver results from decreased hepatic blood flow (due to left-sided heart failure) and from venous congestion (secondary to right-sided heart failure). Long-term hypoxia causes fibrosis of the liver, and the liver progresses to cirrhosis. The prognosis of patients with cardiac cirrhosis depends only on the cardiac disease. It is generally accepted that a small number of these patients develop liver failure and esophageal varices.[6] There has been only one report of HCC due to cardiac cirrhosis (by Ho et al.[7]). However, as they did not check for HCV infection, there is a possibility that this was an underlying factor.

To investigate the relationship between congestive liver disease and HCC, many investigators have studied the hepatocarcinogenesis of BCS, a disorder caused by obstruction of the hepatic vein or the hepatic portion of the IVC. In particular, membranous obstruction (web formation) of the IVC (MOVC) is more frequently associated with HCC than primary hepatic vein thrombosis (classical BCS). Many investigators have reported cases of HCC that arose in patients with MOVC,[8–11] although the etiology is still unknown. Histologically, many Japanese patients with noncancerous liver tissue with MOVC showed cirrhosis, but congestive liver and liver fibrosis have been observed without cirrhosis in South Africa.[12] Matsui et al.[11] reported that HCC developed in 3 of 12 patients with MOVC during a 15-year follow-up period. The hepatitis B viral markers were positive in 1 of these 3 patients with HCC, but the other 2 patients were negative for all viral markers, such as HBV, HCV, hepatitis G virus (HGV), and TT virus (TTV). The noncancerous liver tissues of the HCC patients were cirrhotic. Matsui et al.[11] suggested that chronic congestion of the centrilobulus results in progressive liver-cell necrosis, which is compensated for by liver-cell regeneration, and, hence, increased DNA synthesis.

According to the Fourteenth Report (1996–1997) of the Liver Cancer Study Group of Japan, of HCC patients in Japan, the rate of elderly (over age 60) incidence is as high as 72.9%, while the rate of juvenile (under age 40) incidence is 1.1%. Many investigators have reported that there were some differences between elderly and juvenile HCC, with one of the major differences being the positivity rate for HBsAg. In juvenile HCC, it was as high as 66.7%–91.0%,[1–4] but the rate was only 16.8% of all HCC patients in the Fifteenth Report (1998–1999) of the Liver Cancer Study Group of Japan.[5] In our patient, both HBsAg and antibody to HCV were negative. Histologically, HBsAg and HBcAg were negative according to the immunostaining of the cancerous and the noncancerous liver tissues. Another difference between elderly and juvenile HCC is in the degree of progression. Juvenile HCC more frequently starts with symptoms than the elderly type. The most common symptom is abdominal pain, reported for 53.8%–80.0% of juvenile HCC.[1–4] Because the early stage of juvenile HCC tends to lack subjective symptoms, HCC has already progressed to an advanced stage in juvenile patients when symptoms, such as abdominal pain, appear.

In our patient, both HBV and HCV viral markers were negative. There had been no background factor of liver disease, except for liver congestion associated with

chronic heart failure. This patient represents the first reported case of HCC arising from cardiac cirrhosis in a juvenile. The incidence of HCC in patients with congestive liver disease can be expected to increase in the future, as the survival time is prolonged by advances in treatment for chronic heart failure. Therefore, we would like to emphasize that patients with congestive liver disease should be followed, taking the possibility of HCC occurrence into account.

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臨床薬理

Genotype 1かつ低ウイルス量，あるいはgenotype 2のC型慢性肝炎に対するPEG-インターフェロンα-2bとリバビリン24週併用療法の有効性

―インターフェロンα-2bとリバビリン24週間併用療法との比較―

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索引用語 ■C型慢性肝炎，genotype 2，genotype 1/低ウイルス量，PEG-IFNα-2b，リバビリン併用療法

1 はじめに

C型慢性肝炎（以下，CHC）に対する治療法の進歩に伴い，CHC治療ガイドラインが世界的に次々と変更されてきた．欧米における現在の標準的治療法はPEG-interferon（以下，PEG-IFN）と抗ウイルス薬リバビリンとの併用療法であり，投与期間はgenotype 1では1年間，genotype 2/3では6カ月とされている[1~3]．しかしながら，PEG-IFN＋リバビリン併用療法の有効性が高いgenotype 2/3症例ではさらに短期投与あるいはPEG-IFN量が少量でも有効[4]とされ，一方genotype 1ではウイルス学的反応性が遅れる症例には72カ月の投与が有効との報告[5~7]も出てきている．さらに最近の疫学調査結果によればgenotype 4, 5, 6の感染頻度も上昇してきていること[8]から，欧米におけるCHC治療ガイドラインも早急に改定する必要があると言われている．

わが国でもようやくCHCに対する国際的な標準的治療方法であるPEG-IFN＋リバビリン併用

Hiromitsu KUMADA et al: Efficacy of PEG-interferon α-2b and ribavirin combination therapy for 24 weeks in chronic hepatitis C patients with genotype 1 and low viral load or genotype 2 – open, multicenter, randomized controlled study with interferon α-2b and ribavirin combination therapy –

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療法であるPRG-IFNα-2b（販売名：PEG-Intron, Schering Plough KK, 大阪）とリバビリン（販売名：Rebetol, Schering Plough KK, 大阪）が，2004年12月に一般使用可能となった．しかしながら，欧米とは異なり治療対策の緊急性から臨床研究が難治性のgenotype 1かつ高ウイルス量の症例で先行して実施[9]された関係で適応もこれらの症例に限定されている．

日本におけるCHC治療ガイドラインとしては，日本肝臓学会[10]および厚生労働省厚生労働科学研究費研究班[11]が別個に策定している．後者においてはすでにgenotype 1かつ高ウイルス量例に対する標準治療としてPEG-IFN＋リバビリンが設定されているが，現時点における適応外の「genotype 1かつ高ウイルス量以外の症例」に対する適応拡大要求が当初より強く寄せられていた．その対応として，わが国におけるgenotype分布の検討から，genotype 1かつ低ウイルス量，あるいはgenotype 2の症例におけるPEG-IFN＋リバビリン併用療法の有効性を評価した．PEG-IFNα-2bの投与法は国内既承認と同じ1.5 μg/kg週1回投与とし，リバビリンも国内で承認されている用法・用量を用いた．投与期間は，海外のガイドラインではgenotype 2症例においては6カ月と設定されていることから，これに準拠した．また，PEG-IFN＋リバビリン併用療法の有効性を科学的に評価するには前向き無作為比較試験が必要であることから，国内ではIFN前治療無効例と限定されているが，既承認のIFNα-2b 6MIU＋リバビリンとの比較を行った．さらに，ウイルス陰性化時期別の有効性予測および必要投与期間の検討も行った．

2 検討方法

1. 対象患者

CHC患者のうち，以下の基準をすべて満たす患者を対象とした．①血中HCV-RNAが定量測定（RT-PCR法：アンプリコアHCVモニター）で定量可能（陽性）の患者．または，定量限界以下の場合，定性測定（RT-PCR法：アンプリコアHCV）で陽性の患者，②Genotype，血中HCV-RNA量が以下の基準のうちいずれか一方に合致する患者［1. Genotype 1（Genotype 1a, 1b）の場合，血中HCV-RNA量がRT-PCR法で100 k IU/mL未満，2. Genotype 2（Genotype 2a, 2b）］，③血清ALT値が基準値上限を超える患者［血清ALT基準値上限（三菱化学ビーシーエル）：45 IU/L］，④「慢性肝炎と肝硬変の判別式」[12]の計算結果が負（＜0）で慢性肝炎と判断された患者，⑤クレアチニンクリアランス（Cockcroftらの予測式）が51以上の患者，⑥糖尿病の薬物療法を受けていない患者で，空腹時血糖が110 mg/dL未満の患者（ただし，空腹時血糖110 mg/dL以上126 mg/dL未満の患者については，HbA1cが6.5％未満であれば，登録可能），⑦ヘモグロビン（以下，Hb）濃度≧12 g/dL，⑧好中球数≧1,500 /mm³，⑨血小板数≧10万/mm³，⑩体重が40 kgを超えて100 kg以下の患者，⑪投与開始時から，少なくとも2週間の入院が可能な患者．

なお，以下の基準のいずれかに該当する患者は本治験の対象としなかった．①過去にPolyethylene glycol修飾IFNあるいはリバビリンの投与を受けたことのある患者，②過去にIFN治療歴がある場合は，以下に該当する患者（1. 30週を超える長期投与を受けたことのある患者，2. 登録の時点で，前回のIFN投与終了後90日を経過していない患者），③登録前30日以内に，グリチルリチン・システイン・グリシンを含有する注射用製剤（強力ネオミノファーゲンCなど），小柴胡湯，ウルソデスオキシコール酸の投与を受けた患者，④登録前90日以内に，抗ウイルス剤または抗腫瘍剤の投与あるいは免疫調節療法（ステロイド剤投与，放射線療法を含む）を受けた患者［局所投与および外用剤を除く］，⑤登録前60日以内のスクリーニング検査（集中測定）により，以下に該当する患者（1. HBs抗原陽性の患者，2. 抗核抗体価が160倍以上の患者），⑥肝硬変，肝不全，肝癌を合併している患者またはこれらの既往歴のある患者，⑦自己免疫性肝炎，アルコール性肝障害，薬剤性肝障害などの肝疾患を合併している患者，⑧肝性脳症，食道静脈瘤破裂，腹水の

【PEG/R 群】PEG-IFNα-2b＋リバビリン 併用投与群

PEG-IFNα-2b 1.5 μg/kg **皮下**投与（1 回 / 週）	
リバビリン 600, 800 または 1,000 mg/ 日　経口投与（毎日）	
24 週間	24 週間
投与期間	経過観察期間

【IFN/R 群】IFNα-2b＋リバビリン 併用投与群

IFNα-2b 600 万 IU **筋肉内**投与		
（6 回 / 週）	（3 回 / 週）	
リバビリン 600, 800 または 1,000 mg/ 日　経口投与（毎日）		
2 週間	22 週間	24 週間
投与期間		経過観察期間

図 1　治療法の概略

既往を有する患者，血友病の患者，⑨うつ病あるいは精神神経障害のある患者またはこれらの既往歴のある患者，⑩薬物治療を必要とするてんかん発作のある患者またはその既往歴のある患者，⑪狭心症，心不全，心筋梗塞，高度の高血圧症（拡張期血圧が 120 mmHg 以上）あるいは高度の不整脈のある患者またはこれらの既往歴のある患者，⑫慢性肺疾患の患者またはその既往歴のある患者，⑬自己免疫疾患（クローン病，潰瘍性大腸炎，慢性関節リウマチ，特発性血小板減少性紫斑病，全身性エリテマトーデス，自己免疫性溶血性貧血，強皮症など）のある患者またはこれらの既往歴のある患者，⑭異常ヘモグロビン症（サラセミア，鎌状赤血球性貧血）の患者，⑮悪性腫瘍の患者，⑯薬物療法でコントロール不能な甲状腺機能異常を有する患者，⑰臓器移植を受けた患者（ただし角膜，毛移植を除く），⑱IFN 製剤，ヌクレオシドアナログまたはワクチンなどの生物学的製剤に対して過敏症の既往歴のある患者，⑲投与開始直前に実施するプリック試験において，PEG-IFNα-2b，IFNα-2b に対して特異的な反応の認められた患者，⑳妊娠または授乳中である患者（男性の場合：パートナーが妊娠中の患者），並びに同意取得時から登録時までに測定した血清 HCG 測定結果より妊娠が否定されない患者．

2. 投与方法

1）症例の割り付け

IFN の治療歴が有効性に影響を与える可能性があることから，IFN の治療歴（IFN 未治療，IFN 既治療）別に層別した割り付けを行った．なお，登録にあたっては，中央登録方式とし，施設は割り付けに考慮しなかった．

2）治療法

投与開始日は各群とも両剤の投与を行った．投与開始日におけるリバビリンの投与は朝分より開始した．治療法の概略は図 1 に示したが，投与期間は両群ともに 24 週間，経過観察期間は 24 週間とした．何らかの理由により，治験薬が投与されず残薬を有する場合でも，第 168 日目を超えて PEG-IFNα-2b または IFNα-2b およびリバビリンの投与を行わないこととした．

3）治験薬の投与量・投与方法

（1）PEG-IFNα-2b

1 回投与量は 1.5 μg/kg［1.250 ～ 1.875 μg/kg］とし，週 1 回，24 週間，皮下投与した．注射溶液の調製ならびに投与方法としては，1 バイアル中の注射用凍結乾燥製剤を「日局」注射用水 0.7 mL に溶解し，表 1 に示す投与液量を皮下投与した．投与期間中に体重変動があった場合も，下記の「減量·投与中止規定」に該当しない限り，

表1 体重別の PEG-IFNα -2b の1回あたり投与量

登録時の体重	1回あたりの投与量		
	投与量		投与液量
	(μg/kg)	(μg/回)	(mL)
40 kg を超え 60 kg 以下	1.5 (1.250 ～ 1.875)	75	0.25
60 kg を超え 80 kg 以下		105	0.35
80 kg を超え 100 kg 以下		135	0.45

表2 リバビリンの1日投与量

登録時の体重	1日あたりの投与量 (mg/日)	用法
40 kg を超え 60 kg 以下	600 mg	朝食後：200 mg（200 mg カプセル 1 個） 夕食後：400 mg（200 mg カプセル 2 個）
60 kg を超え 80 kg 以下	800 mg	朝食後：400 mg（200 mg カプセル 2 個） 夕食後：400 mg（200 mg カプセル 2 個）
80 kg を超え 100 kg 以下	1,000 mg	朝食後：400 mg（200 mg カプセル 2 個） 夕食後：600 mg（200 mg カプセル 3 個）

表3 ヘモグロビン，好中球数ならびに血小板数の変動による減量・投与中止規定

項目	対象事象	リバビリンの減量	PEG-IFNα -2b または IFNα -2b の減量	両剤の投与中止	投与量の復帰
ヘモグロビン (g/dL)	8.5 以上，10 未満	●			不可 *
	8.5 未満			●	
好中球数 (/mm³)	500 以上，750 未満		●		可能 **
	500 未満			●	
血小板数 (万 /mm³)	5 以上，8 未満		●		可能 **
	5 未満			●	

* 注意1：ヘモグロビン減少による減量時の初回投与量への復帰

ヘモグロビンの減少により，リバビリンの投与量を減量した場合は，ヘモグロビン値が回復しても投与量の復帰（初回投与量への増量）は行わないものとした．

** 注意2：好中球数 / 血小板数減少による減量時の初回投与量への復帰

好中球数あるいは血小板数の減少により，PEG-IFNα -2b または IFNα -2b を減量した場合は，減量後，好中球数が 750 /mm³ 以上あるいは血小板数が 8 万 /mm³ 以上に増加したときは，治験責任（分担）医師の判断で，PEG-IFNα -2b または IFNα -2b の投与量の復帰（初回投与量への増量）を実施できるものとした．ただし，投与量の復帰に際しては，減量処置後4週間以上の期間をあけることとし，初回投与量への復帰後，再び好中球数あるいは血小板数の減少が認められた場合は，再度本規定にしたがって減量・投与中止を行った．

表4　減量時のPEG-IFN α-2bの1回あたりの投与量

登録時の体重	減量時の1回あたりの投与量		
	投与量		投与液量
	(μg/kg)	(μg/回)	(mL)
40 kgを超え60 kg以下	1.5 (1.250～1.875)	39	0.13
60 kgを超え80 kg以下	↓	54	0.18
80 kgを超え100 kg以下	0.75 (0.650～0.975)	69	0.23

表5　減量時のリバビリンの1日あたりの投与量

登録時の体重	減量時の1日あたりの投与量 (mg/日)	用法
40 kgを超え60 kg以下	600 mg → 400mg	朝食後：200 mg (200 mgカプセル1個) 夕食後：200 mg (200 mgカプセル1個)
60 kgを超え80 kg以下	800 mg → 600mg	朝食後：200 mg (200 mgカプセル1個) 夕食後：400 mg (200 mgカプセル2個)
80 kgを超え100 kg以下	1,000 mg → 600mg	朝食後：200 mg (200 mgカプセル1個) 夕食後：400 mg (200 mgカプセル2個)

原則として初回投与量を変更しないこととした．また，原則としてPEG-IFNα-2bは，投与期間を通じて，毎週，同じ曜日に投与した．

(2) IFN α-2b

IFNα-2bの投与量は600万IUとし，投与開始後2週間は週6回筋肉内投与し，その後の22週間は週3回の頻度で筋肉内投与した．

(3) リバビリン

症例登録時の体重に応じて表2の投与量を初回投与時に設定し，下記の「治験薬の減量・投与中止規定」に該当する場合を除き，以後継続して24週間，連日経口投与（朝・夕，食後）した．ただし，臨床検査実施日で朝食を摂らずに来院する必要がある日は，朝分を空腹時に服用した．また，投与期間中に体重変動があった場合も，「減量・投与中止規定」に該当しない限り，原則として初回投与量を変更しないこととした．

4) 治験薬の減量・中止

投与期間中は，Hb，好中球数ならびに血小板数の変動などを慎重に監視し，下記の基準で「初回投与量」の減量・投与中止を行うこととした．

(1) Hb，好中球数ならびに血小板数の変動による減量・投与中止規定

投与期間中は，臨床検査項目の変動に十分注視し，表3の減量・投与中止規定に該当した場合は速やかに減量・投与中止処置を実施することとした．投与中止規定に該当した場合は，両薬剤の投与を中止することとした．

(2) その他の有害事象による減量・投与中止規定

Hb，好中球数あるいは血小板数の減少以外の有害事象が発現し，投与量の減量，休薬または投与中止を行う必要がある場合，治験担当医師の判断でそれを行うことができることとした．

(3) PEG-IFNα-2bの減量

投与期間中に表3の減量規定に該当した場合，表4に示したように1回投与量を0.75 μg/kg [0.650～0.975 μg/kg] に減量した．また，減量後，減量理由の有害事象が軽快・消失した場合は，治験担当医師の判断で，投与量を初回投与量に復帰させることができることとした．

(4) IFNα-2bの減量

投与期間中，表3の減量規定に該当した場合，投与量を600万IU/回から300万IU/回に減量