Table 5 Comparison between those patients who received adefovir and those who did not

	Administration of adefovir ($n = 36$)		No administration of adefovir ($n = 24$)		P
Age (years) mean ± SD	45.0 ± 10.5		41.3 ± 12.3		NS
Gender: male:female (%male)	29:7 (80.1%)		15:9 (62.5%)		NS
HBeAg-positive patients	25 (69.4%)		19 (79.2%)		NS
Alanine aminotransferase (IU/l)					
Mean ± SD	148.7 ± 202.5		170.0 ± 239.6		NS
Serum HBV DNA (LGE/ml)					
Mean ± SD	7.64 ± 1.03		6.91 ± 1.30		<0.05
YMDD mutation	YIDD	20	YIDD	13	
	YVDD	8	YVDD	4	
	YIDD + YVDD	3	YIDD + YVDD	0	

HBeAg: Hepatitis B e antigen
NS: Not significant
YMDD mutation: YMDD motif of the HBV DNA polymerase
YIDD: Methionine to isoleucine
YVDD: Methioneine to valine

Table 6 Multivariate analysis with Cox proportional hazards model

	Variable	P-value	Risk ratio	95% CI
Virus recrudescence	HBV DNA ≧ 7.0 LGE/ml	0.011	0.466	0.246–0.842
Breakthrough	HBV DNA ≧ 7.7 LGE/ml	0.019	0.444	0.218–0.879

Only variables that achieved statistical significance ($P < 0.05$) are shown
CI: Confidence interval
Breakthrough; administration of adefovir for breakthrough hepatitis due to lamivudine resistance

Table 7 The efficacy of the adefovir add-on treatment

	At 1 year ($n = 36$)	At 2 years ($n = 19$)	At 3 years ($n = 11$)
All patients ($n = 36$)			
ALT Normalization	17 (47.2%)	11 (57.9%)	9 (81.8%)
Loss of HBV DNA	17 (47.2%)	13 (68.4%)	10 (90.9%)
	At 1 year ($n = 25$)	At 2 year ($n = 13$)	At 3 years ($n = 8$)
HBeAg-positive patients ($n = 25$)			
ALT Normalization	12 (48.0%)	7 (53.8%)	7 (87.5%)
Loss of HBV DNA	9 (36.0%)	8 (61.5%)	7 (87.5%)
Loss of HBeAg	3 (12.0%)	5 (38.5%)	6 (75.0%)
HBeAg seroconversion	3 (12.0%)	5 (38.5%)	6 (75.0%)
	At 1 year ($n = 11$)	At 2 year ($n = 6$)	At 3 years ($n = 3$)
HBeAg-negative patients ($n = 11$)			
ALT Normalization	5 (45.5%)	4 (66.7%)	2 (66.7%)
Loss of HBV DNA	8 (72.7%)	5 (83.3%)	3 (100%)

ALT: Alanine aminotransferase
HBeAg: Hepatitis B e antigen

Of these 36 patients who received adefovir, the normalization of ALT levels was 47.2%, 57.9%, and 81.8% at 1, 2, and at 3 years and their loss of serum HBV DNA was 47.2%, 68.4%, and 90.9% at 1, 2, and at 3 years, respectively (Table 7). At the time of this analysis, the median total duration of adefovir treatment was 24 (range = 12–56) months and no adefovir-resistant mutation has been detected in any of these patients.

Fifty patients who did not develop lamivudine resistance showed the normalization of their ALT levels to be 84.0% at 1 year and 86.1%, 92.0%, 90.9%, and 91.9% at 2, 3, 4, and 5 years and the loss of serum HBV DNA was

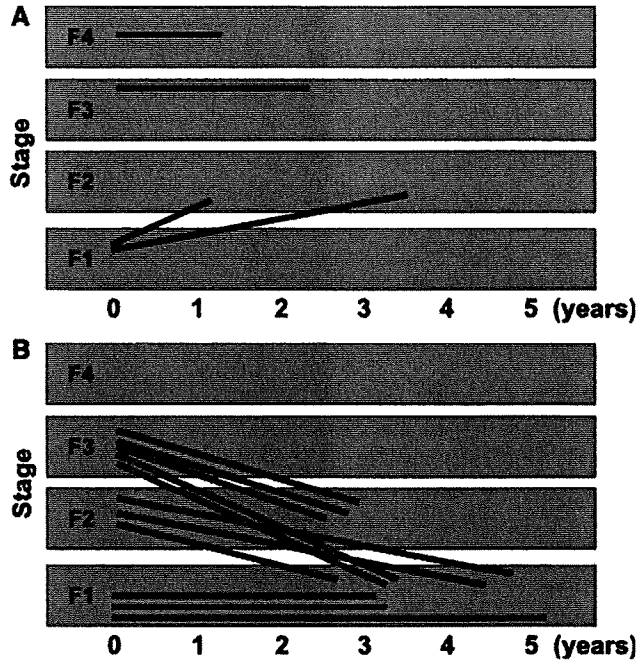

Fig. 1 A follow-up study of liver histology on the patients with chronic hepatitis B treated with lamivudine. (**a**) Four patients whose alanine aminotransferase levels were more than 2 times the upper limit of normal showed no improvement in liver fibrosis at the second liver biopsy. (**b**) Nine patients whose alanine aminotransferase levels were less than 2 times the upper limit of normal demonstrated an improvement in liver fibrosis at the second liver biopsy even if the serum HBV DNA was positive. Another 3 patients whose degree of fibrosis was diagnosed to be F1 at the first liver biopsy showed no change in liver fibrosis

92.0% at 1 year and 94.4%, 96.0%, 95.5%, and 100% at 2, 3, 4, and 5 years, respectively. The results were good for the patients who did not develop lamivudine resistance.

Fifteen patients who were treated with lamivudine underwent a repeat liver biopsy. The changes in stages of liver fibrosis are shown in Fig. 1. Figure 1a shows the group of the patients whose ALT levels were more than two times the upper limit of normal. Figure 1b shows the group of the patients whose ALT levels were less than two times the upper limit of normal. A progression in the fibrosis stage was observed in two patients and an improvement in the fibrosis stage was observed in 8 patients. Even if the serum HBV DNA level showed positive values, the degree of liver fibrosis improved on the basis of the findings of a second liver biopsy in the patients who were treated with lamivudine if the ALT level was less than two times the upper limit of normal.

To clarify the long-term efficacy of the lamivudine treatment of Japanese patients with chronic hepatitis B with or without adefovir add-on treatment of breakthrough hepatitis due to lamivudine-resistance, we investigated the efficacy of the lamivudine treatment by our new approach. Table 2 shows those patients who had an efficacy of adefovir salvage treatment of breakthrough hepatitis and were excluded from the efficacy group of the patients with lamivudine treatment. In addition, in Table 8, the patients who had the efficacy of adefovir add-on treatment were included in the efficacy group for 5-year period since lamivudine treatment was started. These results included all the patients

Table 8 The efficacy of lamivudine treatment with or without adefovir add-on ($n = 110$)

	At 1 year ($n = 110$)	At 2 years ($n = 94$)	At 3 years ($n = 79$)	At 4 years ($n = 63$)	At 5 years ($n = 44$)
All patients ($n = 110$)					
ALT normalization	77 (70.0%)	55 (58.5%)	48 (60.8%)	42 (66.7%)	27 (61.4%)
Loss of HBV DNA	80 (72.7%)	55 (58.5%)	43 (54.4%)	36 (57.1%)	27 (61.4%)
	At 1 year ($n = 67$)	At 2 years ($n = 61$)	At 3 years ($n = 54$)	At 4 years ($n = 43$)	At 5 years ($n = 31$)
HBeAg-positive patients ($n = 67$)					
ALT normalization	48 (71.6%)	34 (55.7%)	29 (53.7%)	26 (60.5%)	17 (54.8%)
Loss of HBV DNA	47 (70.1%)	27 (44.3%)	24 (44.4%)	21 (48.8%)	16 (51.6%)
Loss of HBeAg	20 (29.9%)	20 (32.8%)	20 (37.0%)	18 (41.9%)	14 (45.2%)
HBeAg seroconversion	16 (23.9%)	19 (31.1%)	19 (35.2%)	18 (41.9%)	14 (45.2%)
	At 1 year ($n = 43$)	At 2 years ($n = 33$)	At 3 years ($n = 25$)	At 4 years ($n = 20$)	At 5 years ($n = 13$)
HBeAg-negative patients ($n = 43$)					
ALT Normalization	29 (67.4%)	21 (63.6%)	19 (76.0%)	16 (80.0%)	10 (76.9%)
Loss of HBV DNA	33 (76.7%)	28 (84.8%)	19 (76.0%)	15 (75.0%)	11 (84.6%)

ALT: Alanine aminotransferase

HBeAg: Hepatitis B e antigen

with or without lamivudine-resistance and all the patients with or without adefovir add-on therapy. In all these patients, including those who were HBeAg-positive and those who were HBeAg-negative, the normalization of the ALT levels was 61.4% at 5 years since lamivudine treatment was started (Table 8). The loss of serum HBV DNA was 61.4% at 5 years since the lamivudine treatment was started. In the HBeAg-positive patients, the normalization of the ALT levels, the loss of serum HBV DNA, and HBeAg seroconversion was 54.8%, 51.6%, and 45.2% at 5 years, respectively, since the lamivudine treatment was started. In the HBeAg-negative patients, the normalization of the ALT levels and the loss of serum HBV DNA was 76.9% and 84.6% at 5 years, respectively, since the lamivudine treatment had been started.

Discussion

We herein describe the long-term efficacy of lamivudine treatment over a 5-year period. In all patients, including those who were HBeAg-positive and those who were HBeAg-negative, the normalization of ALT levels and the loss of serum HBV DNA gradually decreased to 36.4% and 31.8% at 5 years, respectively. The efficacy of lamivudine treatment was only about 30%. This result showed that lamivudine monotherapy could not improve the long-term outcomes in the patients with chronic hepatitis B. However, in the HBeAg-negative patients, the effects of lamivudine treatment were maintained for long-term. Although we assessed only a small number of patients in this study, we consider lamivudine monotherapy to be an effective treatment modality for patients with HBeAg-negative chronic hepatitis B to improve the long-term outcomes. On the other hand, because in the HBeAg-positive patients, virus recrudescence was more frequently observed than in the HBeAg-negative patients, the efficacy of the lamivudine treatment for 5 years was only about 20%. HBeAg-positive patients who demonstrated a high value of serum HBV DNA level and a low ALT level at the start of the lamivudine therapy showed a significant correlation with an increase in the occurrence of lamivudine-resistant mutations. Our Cox proportional hazards model analysis shows that to obtain serum HBV DNA levels of less than 7.7 LGE/ml before lamivudine treatment might associate with good outcomes in those patients. These patients are therefore recommended to reduce their serum HBV DNA levels before commencing lamivudine treatment and using interferon α or other therapeutic options [5, 6, 12]. Further large-scale and long-term studies are needed to confirm these observations.

In our study, although most virus recrudescence was observed within 1 year after the lamivudine treatment, 1 patient demonstrated virus recrudescence at 5 years after the lamivudine treatment was started. This is a very important finding and we must therefore carefully monitor the serum HBV DNA levels of the patients even if they show a good long-term response to the lamivudine treatment. Thirty-six of the patients with virus-recrudescence had adefovir added to the lamivudine regimen for the treatment of breakthrough hepatitis. However, the other 24 patients (40%) did not need adefovir to be added to the treatment regimen because they did not demonstrate hepatitis. In our study, even if the serum HBV DNA level was found to be positive, a histologic improvement was obtained in patients with ALT levels that were under 2 times the upper limit of normal at the time of a second liver biopsy. We thus consider that the normalization of ALT levels might therefore be more important than achieving a negative serum HBV DNA state to achieve a remission of liver disease. However, the risks of hepatocellular carcinoma and cirrhosis in the patients with chronic hepatitis B have recently been reported to be related to the serum HBV DNA levels [13, 14]. Our 24 patients who did not have adefovir added to the treatment regimen, even if they had virus recrudescence, all required a long-term follow-up to monitor whether or not they might progress to either cirrhosis or hepatocellular carcinoma.

Some reports have shown that among patients who experienced HBeAg seroconversion during the lamivudine treatment, the durability of the response after the cessation of therapy ranged from 38% to 77% [15–17]. Because lamivudine therapy still continues to be administered in Japan to avoid any posttreatment flare-ups of hepatitis [18–20], our patients continue to receive lamivudine treatment; therefore, our data are not comparable with other studies involving a cessation of treatment.

Previous studies reported that in patients receiving adefovir, the HBV DNA level decreased by 3.5 to 3.9 $\log_{10}$ from the baseline level [21, 22]. In addition, studies from Asia reported that patients with lamivudine resistance have been treated with adefovir either in monotherapy or in combination with lamivudine without any significant differences between the 2 regimens [23, 24]. On the other hand, studies from Europe reported that adding adefovir to lamivudine for the treatment of patients with lamivudine-resistant HBeAg-negative chronic hepatitis B maximizes the anti-viral efficacy because of the absence of viral resistance [25, 26]. In Japan, adefovir has been used as an additional therapy to suppress viral replication with lamivudine-resistant mutations. In all the patients investigated in our study, adefovir was added to the lamivudine regimen. However, no virologic or biochemical breakthrough was reported because no adefovir-resistance occurred in any of our patients. We thus considered that the condition of no adefovir-resistance might have occurred as all

our patients received adefovir in combination with lamivudine.

To clarify the long-term efficacy of the lamivudine treatment of Japanese patients with chronic hepatitis B, we investigated the efficacy of lamivudine treatment on the basis of our new approach. In all the patients with or without lamivudine resistance and with or without adefovir add-on treatment, the normalization of the ALT levels was 61.4% and the loss of serum HBV DNA was 61.4% after the 5-year period of treatment from the time of lamivudine-treatment. This study is the first report to clarify the long-term efficacy of lamivudine treatment either with or without adefovir from the time the lamivudine treatment was started. Our study suggests that even in patients with chronic hepatitis B who have a high HBV DNA level and/or who are HBeAg-positive, the combination therapy of lamivudine and adefovir appears to be an effective treatment modality. This is a retrospective observational study with a relatively heterogeneous patient population. Although this study has its limitation, it represents the real situation for the treatment of chronic hepatitis B in Japan. The advantages of this study, such as being a single-center study, where all tests were performed in the same laboratory using the same methods, are thus considered to outweigh these limitations. These preliminary observations need to be validated in future studies with a larger number of patients.

In conclusion, although the efficacy of lamivudine is limited because of breakthrough hepatitis, adefovir was used as a salvage treatment of lamivudine resistance in patients with chronic hepatitis B. Therefore, the use of lamivudine for the treatment of Japanese patients with chronic hepatitis B with or without lamivudine-resistance is thus considered to be a useful treatment modality for obtaining long-term virologic and biochemical responses.

References

1. Lee WM. Hepatitis B virus infection. N Engl J Med 1997; 337:1733–45.
2. World Health Organization. Fact sheet WHO/204. Hepatitis B. Geneva, Switzerland: World Health Organization; 2003.
3. Beasley RP. Hepatitis B virus: the major etiology of hepatocellular carcinoma. Cancer 1988;61:1942–56.
4. Hepatitis B fact sheet. Atlanta, GA: National Center for Infectious Disease, Centers for Disease Control and Prevention; 2004.
5. Wong DK, Cheung AM, O'Rourke K, Naylor CD, Detsky AS, Heathcote J. Effect of alpha-interferon treatment with hepatitis B e antigen-positive chronic hepatitis B. A meta-analysis. Ann Intern Med 1993;119:312–23.
6. Manesis EK, Hadziyannis SJ. Interferon α treatment and retreatment of hepatitis B e antigen-negative chronic hepatitis B. Gastroenterology 2001;121:101–9.
7. Liaw YF, Chien RN, Yeh CT, Tsai SL, Chu CM. Acute exacerbation and hepatitis B virus clearance after emergence of YMDD motif mutation during lamivudine therapy. Hepatology 1999;30:567–72.
8. Hadziyannis SJ, Papatheodoridis GV, Dimou E, Laras A, Papaioannou C. Efficacy of long-term lamivudine monotherapy in patients with hepatitis B e antigen-negative chronic hepatitis B. Hepatology 2000;32:847–51.
9. Liaw YF. Impact of YMDD mutations during lamivudine therapy in patients with chronic hepatitis B. Antivir Chem Chemother 2001;12:67–71.
10. Peters M, Hann HWH, Martin P, Heathcote EJ, Buggisch P, Rubin R, et al. Adefovir dipivoxil alone or in combination with lamivudine in patients with lamivudine-resistant chronic hepatitis B. Gastroenterology 2004;126:91–101.
11. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Long-term therapy with adefovir dipivoxil for HBeAg-negative chronic hepatitis B. N Engl J Med 2005;352:2673–81.
12. Schalm SW, Heathcote J, Cianciara J, Farrell G, Sherman M, Willems B, et al. Lamivudine and alpha interferon combination treatment of patients with chronic hepatitis B infection: a randomised trial. Gut 2000;46:562–8.
13. Iloeje UH, Yang HI, Su J, Jen CL, You SL, Chen CJ. Predicting cirrhosis risk based on the level of circulating hepatitis B viral load. Gastroenterology 2006;130:678–86.
14. Chen CJ, Yang HI, Su J, Jen CL, You SL, Lu SN, et al. Risk of hepatocellular carcinoma across a biological gradient of serum hepatitis B virus DNA level. JAMA 2006;295:65–73.
15. Lee KM, Cho SW, Kim SW, Kim HJ, Hahm KB, Kim JH. Effect of virological response on post-treatment durability of lamivudine-induced HBeAg seroconversion. J Viral Hepat 2002;9:208–12.
16. Dienstag JL, Cianciara J, Karayalcin S, Kowdley KV, Willems B, Plisek S, et al. Durability of serologic response after lamivudine treatment of chronic hepatitis B. Hepatology 2003;37:748–55.
17. van Nunen AB, Hansen BE, Suh DJ, Lohr HF, Chemello L, Fontaine H, et al. Durability of HBeAg seroconversion following antiviral therapy for chronic hepatitis B: relation to type of therapy and pretreatment serum hepatitis B virus DNA and alanine aminotransferase. Gut 2003;52:420–4.
18. Ide T, Kumashiro R, Suzuki H, Tanikawa K, Sata M. Two-year follow-up study after treatment with lamivudine for chronic hepatitis B: seven cases reported. Hepatol Res 2000;17:197–204.
19. Honkoop P, de Man RA, Niesters HG, Zondervan PE, Schalm SW. Acute exacerbation of chronic hepatitis B virus infection after withdrawal of lamivudine therapy. Hepatology 2000; 32:635–9.
20. Bonacini M, Kurz A, Locarnini S, Ayres A, Gibbs C. Fulminant hepatitis B due to a lamivudine-resistance mutant of HBV in a patient coinfected with HIV. Gastroenterology 2002;124:244–5.
21. Hadziyannis SJ, Tassopoulos NC, Heathcote EJ, Chang TT, Kitis G, Rizzetto M, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-negative chronic hepatitis B. N Engl J Med 2003;348:800–7.
22. Marcellin P, Chang TT, Lim SG, Tong MJ, Sievert W, Shiffman ML, et al. Adefovir dipivoxil for the treatment of hepatitis B e antigen-positive chronic hepatitis B. N Engl J Med 2003;348: 808–16.
23. Dai CY, Chuang WL, Hsieh MY, Lee LP, Huang JF, Hou NJ, et al. Adefovir dipivoxil treatment of lamivudine-resistant chronic hepatitis B. Antivir Res 2007;75:146–51.

24. Fung J, Lai CL, Yuen JC, Wong DK, Tanaka Y, Mizokami M, et al. Adefovir dipivoxil monotherapy and combination therapy with lamivudine for the treatment of chronic hepatitis B in an Asian population. Antivir Ther 2007;12:41–6.
25. Rapti I, Dimou E, Mitsoula P, Hadziyannis SJ. Adding-on versus switching-to adefovir therapy in lamivudine-resistant HBeAg-negative chronic hepatitis B. Hepatology 2007;45:307–13.
26. Manolakopoulos S, Bethanis S, Koutsounas S, Goulis J, Vlachogiannakos J, Christias E, et al. Long-term therapy with adefovir dipivoxil in hepatitis B e antigen-negative patients developing resistance to lamivudine. Aliment Pharmacol Ther 2008;27: 266–73.

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＜速　報＞

肝硬変に対する瀉血療法による血清AFP値の低下

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緒言：瀉血療法はC型慢性肝炎においてALTを低下させ，さらに発癌抑制効果を持つことが報告されている[1]．AFPは肝癌の診断に有用な腫瘍マーカーであるが，肝硬変における高AFP血症は肝癌発生の危険因子と考えられている[2]．そこで我々は肝硬変に対する瀉血療法中のAFP値の変化に着目した．

対象と方法：6カ月以上の瀉血療法を行った肝硬変患者9例を対象とした．平均年齢は53.9歳（45-62歳）で全例男性であった．HCV症例7例，NASH症例2例で，ラジオ波焼灼術による肝癌の治療歴のある2例を含むが，これらは治療後の造影CTで焼灼が十分であることが確認され，その後も6カ月以上再発を認めていない．瀉血は1回400 mlで月1回のペースで行い，治療前と開始6カ月または12カ月の検査値の比較を行った．

成績：9症例の検査値（平均±SE）を治療前後で比較すると，ALTは111.4±22.9 IU/lから76.1±19.4 IU/lと低下傾向を認めた．AFPは102.0±72.0 ng/mlから35.5±19.2 ng/mlとフェリチンと同様に有意に低下した．なお，2例ではALTが瀉血後に上昇していたが，AFPはこれらの症例も含む全例で低下していた（Table 1）．

考察：肝硬変においては強力ネオミノファーゲンC投与によってALTを低下させた場合でもAFPの有意な低下はみられないとの報告がある[3]．瀉血療法によるALTの低下は，十分な除鉄後に始まるが，本9症例のAFPの低下は治療早期よりみられ，ALTよりもむしろフェリチンと平行しており，抗炎症ではなく除鉄による直接的な効果が推測される．一方，インターフェロンα（IFNα）はALTの低下だけでなくAFPを低下させるが，IFNαは直接的な発癌抑制作用を持つため，このAFPの低下は発癌抑制につながると考えられている[3]．瀉血療法も著明にAFPを低下させたことから，IFNαと同様に抗炎症以外の発癌抑制効果を持つ可能性がある．現在，メカニズムの解明と長期観察を行っている．

Table 1 Changes of serum ALT, ferritin and AFP levels during phlebotomy therapy

age	gender	etiology	follow up period/ total volume of phlebotomy	ALT (IU/L)		ferritin (μg/dL)		AFP (ng/mL)	
				before	after	before	after*	before	after*
61	M	HCV**	6 months/2400 L	87	143	323	21	670.9	110.3
60	M	HCV	12 months/4800 L	73	34	2382	22	109.9	12.3
44	M	HCV**	12 months/4800 L	43	59	665	29	56.1	16.3
51	M	HCV	12 months/4800 L	160	76	871	28	40.4	6.0
56	M	NASH	6 months/2400 L	61	55	272	8	11.2	6.8
45	M	HCV	12 months/4800 L	131	96	842	19	11.1	7.2
59	M	HCV	6 months/2400 L	156	19	2100	31	7.8	2.0
47	M	NASH	12 months/4800 L	249	212	1359	36	6.3	5.3
62	M	HCV	12 months/4800 L	43	30	418	10	4.4	3.6

*$p < 0.01$ compared to the data before therapy

**have a history of curative therapy for hepatocellular carcinoma

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文献：1) Kato J, Miyanishi K, Kobune M, et al. J Gastroenterol 2007; 42: 830—836　2) Ikeda K, Saitoh S, Koida I, et al. Hepatology 1993; 18: 47—53　3) Murashima S, Tanaka M, Haramaki M, et al. Dig Dis Sci 2006; 51: 808—812

英文要旨

Reduction of serum alpha-fetoprotein levels by phlebotomy in patients with cirrhosis

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Phlebotomy is a possible therapy for cirrhosis. We have performed phlebotomy in 9 cirrhotic patients consisting of 7 with HCV infection and 2 with non-alcoholic steatohepatitis. Two of these patients have a history of curative therapy for hepatocellular carcinoma. All patients who received phlebotomy had a marked reduction of serum alpha-fetoprotein levels as well as transaminase levels. Although suppression of hepatocellular damage and liver regeneration cause a reduction in AFP, other mechanisms may also be behind such a reduction. However an elevated serum AFP level is thought to be an important predictor of hepatocarcinogenesis, suggesting that phlebotomy may have a strong potential in the suppression of hepatocellular carcinoma.

Key words: phlebotomy, alpha-fetoprotein, liver cirrhosis

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C型慢性肝炎に対するペグインターフェロンα-2a単独療法の治療効果と治療効果予測因子の検討

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＜原　著＞

C型慢性肝炎に対するペグインターフェロンα-2a単独療法の治療効果と治療効果予測因子の検討

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要旨：C型慢性肝炎の抗ウイルス療法は，ペグインターフェロンとリバビリン併用療法が主流であるが，リバビリンの副作用のため併用療法困難な症例ではペグインターフェロンα-2a（PEG-IFN）単独療法も考慮される．今回我々は，PEG-IFN単独療法の治療効果とSustained virological response（SVR）予測因子について検討を行った．PEG-IFN単独療法を行った84例のうち，SVRは56例（ITT解析，66.7%）であった．治療前のSVR予測因子は，若年例（$p=0.0464$），肝線維化が軽度の症例であり（$p=0.0002$），治療後の因子は，治療開始後4週以内のHCV RNA定性陰性化を来たすRapid virological response（RVR）（$p<0.0001$）であった．多変量解析では，RVRがSVR予測因子であった（オッズ比：17.2，$p=0.0001$）．また，セロタイプ1型では，治療前HCV RNA量が400 KIU/ml未満（$p=0.037$），セロタイプ2型では500 KIU/ml未満（$p=0.047$）であればSVRを来たす可能性が高く，PEG-IFN単独療法のよい適応であると考えられた．

索引用語：C型慢性肝炎　ペグインターフェロン単独療法
SVR（Sustained virological response）
RVR（Rapid virological response）　肝線維化

はじめに

C型慢性肝炎の世界標準治療は現在，ペグインターフェロンとリバビリン併用療法である[1)2)]．しかしながら，高齢者や腎障害，貧血を合併する患者にはリバビリンの副作用が強く発現するため，リバビリンの併用が困難な例も多い[3)]．また，挙児希望患者においてもリバビリンの催奇形性が問題となる．このようなリバビリン併用困難なC型慢性肝炎患者の抗ウイルス療法では，インターフェロン単独療法が選択されると考えられる．現在，本邦では従来のインターフェロンとペグインターフェロンα-2a（以下PEG-IFN）が保険適用を受けている．

PEG-IFNは投与初期の副作用が少なく，高齢者であっても十分耐用可能と考えられている[4)]．しかしながら，本邦におけるペグインターフェロン単独治療の治療成績に関する報告は少ない[5)-7)]．そこで，今回，当科ならびに当科関連施設におけるC型慢性肝炎患者に対するPEG-IFN単独療法の治療成績と治療効果に影響を及ぼす因子を解析し，本邦におけるPEG-IFN単独療法の治療適応を明らかにする目的で本研究を行った．

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Table 1 Baseline characteristics of the patients studied

Subjects	Overall (n = 84)	Serotype 1 (n = 20)	Serotype 2 (n = 63)
Gender			
Male : Female	42 : 42	9 : 11	33 : 30
Fibrosis stage*			
F0/F1/F2	2/36/23	0/8/5	2/27/18
F3/F4/	10/4	5/2	5/2
(No Bx**)	(10)	(1)	(9)
Age (yrs)	49.7±14.4	57.8±9.6	47.6±14.7
ALT (IU/l)	95.4±85.6	79.9±61.8	96.1±78.1
Platelet counts (×10⁴/ul)	19.0±6.97	17.8±5.74	19.7±7.25
BMI*** (kg/m²)	23.1±3.45	23.0±3.09	23.2±3.63
HCV RNA (KIU/ml)	655±1060	319±511	761±1190

Values are means±SD

* Fibrosis stage was evaluated by New Inuyama's classification.

** No Bx; The cases which liver biopsy was not performed before treatment.

*** BMI; Body mass index

対象と方法

当科ならびに当科関連施設において，2004年1月から2005年9月までに，C型慢性肝炎治療としてPEG-IFN単独療法を施行された84症例を対象とした．HBs抗原陽性患者，抗ミトコンドリア抗体陽性患者，アルコール性肝炎，自己免疫性肝炎合併患者は本研究の対象から除外された．PEG-IFN（PEGASYS®，Rosch, Switzerland，日本販売元：中外製薬（株），東京）は180 μgを週1回皮下投与し，48週投与を原則とした．治療開始後，好中球750/mm³未満，血小板数5万/mm³未満となればPEG-IFNを90μg/週に減量し，好中球500/mm³未満，血小板数2.5万/mm³未満，ヘモグロビン値8.5 g/dl未満となればPEG-IFN投与は中止とした．PEG-IFN治療開始前に同意を得られた75例では，エコーガイド下の肝生検を施行し（16 G-automatic needleを使用），当院病理医により新犬山分類に基づいた肝線維化の評価を行った[8]．治療終了6カ月後の血清HCV RNA陰性が得られた症例をsustained virological response (SVR)とし，SVR率を求めた．また，SVR症例とnon-SVR症例の二群間で，年齢，性，投与開始前の血清ALT値，血小板数，Body mass index（BMI），肝線維化，HCV RNAセロタイプ，HCV RNA量（アンプリコア，ハイレンジ法）を比較した．さらに，セロタイプ1型，2型各々の症例におけるSVR例とnon-SVR例の二群間での検討を加えた．次に，投与開始後の血清HCV RNA定性陰性化時期別のSVR率を求めた．また，PEG-IFN治療中の血清ALT値の変動についても検討した．

二群間の有意差検定については，フィッシャーの直接確率法を用い，平均±SDで比較する群ではt-検定を用いた．傾向検定はCochran-Armitage検定を行った．多変量解析は多重ロジスティック回帰分析を行った．いずれもp値0.05未満を有意差ありとした．

結　果

当科ならびに関連施設においてPEG-IFN単独療法を受けた症例は84例であった．その内訳は，男性42例，女性42例，平均年齢49.7歳であった．HCVセロタイプ別では，1型20例，2型63例，分類不能1例であった（Table 1）．治療終了後24週時点での効果判定が可能であった症例は全体で81例であり，そのうちSVR例は56例（66.7%），non-SVR例は25例（29.7%）であった．治療開始後，副作用や，他疾患の加療に専念する等の理由のためPEG-IFN療法を中止され，中止後6カ月後の効果判定が不明であった例（Drop out）は3例（3.6%）であった（Fig. 1）．全体における治療結果を踏まえ，SVR群とnon-SVR群における患者背景をTable 2に示す．治療前における因子の検討について，SVR群の平均年齢はnon-SVR群に比して有意に低かった（p=0.0464）．また，SVR群では，non-SVR群に比較して，治療前の血小板数が高く，BMIは低値であり，HCV RNA量（アンプリコア法）が低い傾向を認めたが，両

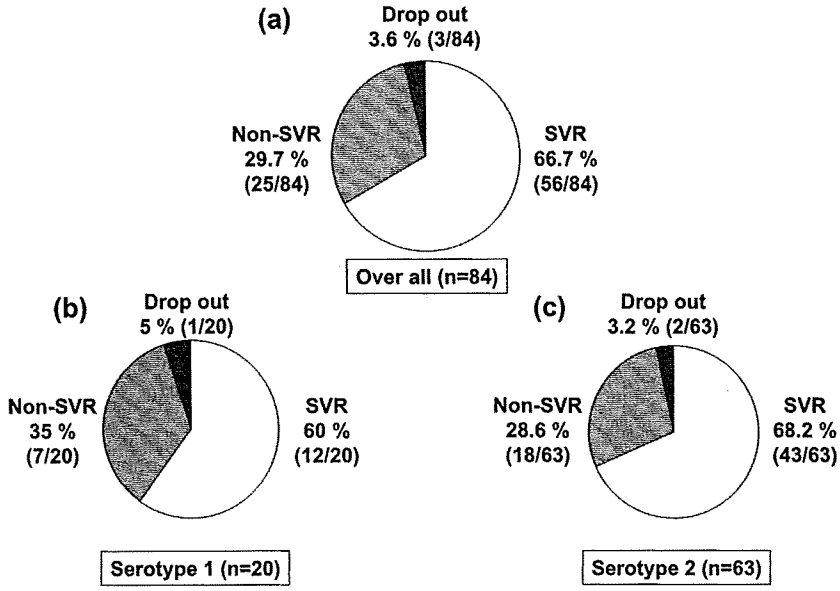

Fig. 1 The outcome of the PEG-IFN monotherapy is shown. (a) The study included a total of 84 patients. SVR * was achieved in 56 cases (66.7%), while non-SVR was observed in 25 cases (29.7%). (b) Among 20 patients with serotype 1, SVR was achieved in 12 cases (60%). (c) Among 63 patients with serotype 2, SVR was achieved in 43 cases (68.2%). (Intention to treat analysis)
* SVR; Sustained virological response

群間で有意差を認めなかった．また，HCVセロタイプにおいても両群間で有意差を認めなかった．Drop out症例を除いた肝線維化とSVR率の検討では，F0＋F1の軽度線維化群，F2＋F3の線維化進行群，F4の肝硬変群，の三群間で傾向検定を行ったところ，SVR率に有意差を認めた(Fig. 2，$p=0.0002$)．また，F4ステージ（肝硬変例）は3例あったが，1例もSVRに至らなかった．

次に，HCVセロタイプ別におけるSVR群，non-SVR群間の検討を示す．セロタイプ1型では，治療前HCV RNA量で有意差を認めたが(Table 3，$p=0.0048$)，セロタイプ2型では各項目で明らかな有意差を認めなかった（Table 4）．また，HCV RNA量からみたSVR率では，セロタイプ1型かつ低ウイルス量（100 KIU/ml以下）症例のSVR率は85.7%（7例のうち6例），セロタイプ2型かつ低ウイルス量症例では90.5%（21例のうち19例）と，いずれもHCV RNA量100 KIU/ml以下の低ウイルス症例において高いSVR率を示した（Table 5）．

治療開始後におけるHCV RNA定性陰性化時期の検討では，治療開始後4週以内にHCV RNA定性陰性化が得られたRapid virological response（RVR）群では，5週～12週の間にHCV RNA定性陰性化が得られたEarly virological response（EVR）群より，有意にSVR率が高率であった(Fig. 3，89.5% 対 61.5%，$p=0.028$)．また，治療開始後12週目以降にHCV RNA定性陰性化を認めた群では，1例もSVRに至らなかった．

これらの結果を踏まえ，多変量解析を行ったところ，SVRに寄与する因子としては，RVRが最も有意な予測因子であった（オッズ比：17.2，95%CI 4.38–84.5，$p=0.0001$）．また，RVRを来した症例がSVRである感度は82.3%，特異度は78.7%，陽性的中率（Positive predictive value：PPV）は89.6%，陰性的中率（Negative predictive value：NPV）は66.7%であった（Table 7）．

PEG-IFNの副作用としての血清ALT値上昇の頻度を明らかにするために，SVR症例の血清ALT値の変動を検討した．SVR症例56例中，治療中の血清ALT値を経時的に確認できた例は23例あったが，そのうち10例（43.5%）では，PEG-IFN投与中に血清ALT値が正常上限値（30 IU/l）よりも高値で推移した（Fig. 4）．

Table 2 Results and characteristics post PEG-IFN monotherapy

	SVR (n = 56)	non-SVR (n = 25)	*p* value
Male : Female	29 : 27	13 : 12	0.5885
Age (yrs)	50.5 ± 14.2	54.4 ± 11.9	0.0464
ALT (IU/*l*)	99.2 ± 88.6	92.1 ± 81.2	0.7303
Platelet counts ($\times 10^4$/u*l*)	19.9 ± 6.8	17.7 ± 7.0	0.1742
BMI (kg/m^2)	22.7 ± 3.2	24.1 ± 3.9	0.1202
HCV RNA (KIU/m*l*)	532 ± 998	947 ± 1204	0.1190
HCV Serotype			
1/2/unknown	12/43/1	7/18/0	0.5787
Fibrosis stage			
F0 + 1/F2 + 3/F4	30/20/0	7/12/3	
(No Bx*)	(6)	(3)	
RVR**	83.0% (44/53)	21.7% (5/23)	< 0.0001

* No Bx; The cases which liver biopsy was not performed before treatment.
** RVR indicates rapid virological response, which is defined as HCV RNA PCR-seronegative at week 4 of treatment.

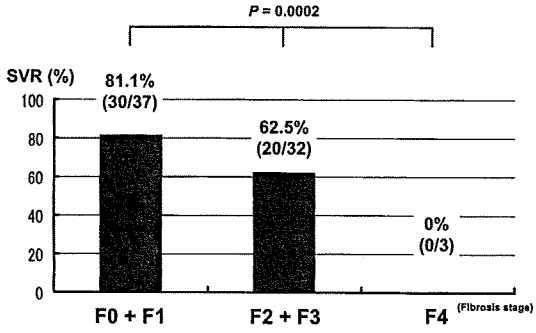

Fig. 2 The rate of SVR at different stages of liver fibrosis progression. Cochran-Armitage trend test analysis showed significant differences in the SVR coefficient among nominal to mild fibrosis (F0 + F1), moderate to severe fibrosis (F2 + F3) and cirrhosis (F4) (p = 0.0002). Drop out cases were excluded (n = 3).

考　察

C型慢性肝炎では，本邦，海外において現在ではペグインターフェロンとリバビリンの併用療法が標準的治療法である．Friedらは，C型慢性肝炎高ウイルス量の症例に対するPEG-IFNα-2a（180 μg/週）とリバビリン（1000–1200 mg/日）の48週併用療法群のSVRは，PEG-IFNα-2a（180 μg）48週単独療法群のSVRと比較して，有意に高率であると報告している（56% 対 29%）[9]．しかしながら実際の臨床の現場ではリバビリンの併用が困難である症例も散見されるため，この様な症例に対してはインターフェロン単独療法あるいは，ペグインターフェロン単独療法が選択される．PEG-IFNは副作用が軽く，週1回の投与で済むため，本邦においては第1選択になると思われる．

Zeuzemらはペグインターフェロンα-2a単独療法におけるSVRに寄与する独立因子として，40歳以下，非肝硬変の状態，体表面積2 m^{2}以下，PEG-IFNα-2aによる治療，治療前ウイルス量2百万コピー/m*l*，ALT値が正常の3倍以上，HCV genotype1以外を挙げ[10]，Leeらは，Zeuzemらの結果に加え，開始時の体重が85 kg以下，HAI score 10点以上のものを挙げている[11]．我々の検討では，SVR群とnon SVR群では，治療前の年齢においてのみ有意差を認めたが，HCV RNAセロタイプ，血小板数，BMI，ALT値，HCV RNA量においては有意差を認めなかった．ジェノタイプ1型かつ高ウイルス量の症例ではPEG-IFN単独療法におけるSVRが低いことが報告されているが[5]，本研究においてHCVセロタイプはSVR予測因子とならなかった．このため，セロタイプ別での検討を加えたところ，まず治療前のセロタイプ1型と2型の各因子を比較すると，セロタイプ1型のSVR群では比較的治療前のHCV RNA量が低かったこと（平均：121 KIU/m*l*）が挙げられ，セロタイプ1型のSVR率上昇と，セロタイプ2型のSVR

Table 3 Results and characteristics between SVR and non-SVR groups in serotype 1

	SVR (n = 12)	non-SVR (n = 7)	*p* value
Male : Female	7 : 5	2 : 5	0.3498
Age (yrs)	56.5 ± 9.17	60.0 ± 11.1	0.4445
ALT (IU/*l*)	73.3 ± 44.6	91.1 ± 87.0	0.5396
Platelet counts ($\times 10^4$/u*l*)	17.4 ± 5.86	18.4 ± 5.93	0.0821
BMI (kg/m^2)	23.2 ± 3.5	22.6 ± 2.2	0.7180
HCV RNA (KIU/m*l*)	121 ± 113	659 ± 738	0.0048
Fibrosis stage			
F0 + 1/F2 + 3/F4	7/5/0	1/5/1	
(No Bx*)	(0)	(0)	
RVR**	81.8% (9/11)	0% (0/6)	0.0002

* No Bx; The cases which liver biopsy was not performed before treatment.
** RVR indicates rapid virological response, which is defined as HCV RNA PCR-seronegative at week 4 of treatment.

Table 4 Results and characteristics between SVR and non-SVR groups in serotype 2

	SVR (n = 43)	non-SVR (n = 18)	*p* value
Male : Female	22 : 21	11 : 7	0.5778
Age (yrs)	45.6 ± 15.4	52.2 ± 11.9	0.1038
ALT (IU/*l*)	97.5 ± 95.2	92.5 ± 81.4	0.8220
Platelet counts ($\times 10^4$/u*l*)	20.5 ± 6.99	17.4 ± 7.63	0.1107
BMI (kg/m^2)	22.7 ± 3.1	24.5 ± 4.3	0.0687
HCV RNA (KIU/m*l*)	636 ± 1113	1059 ± 1344	0.2178
Fibrosis stage			
F0 + 1/F2 + 3/F4	22/15/0	6/7/2	
(No Bx*)	(6)	(3)	
RVR**	83.3% (35/42)	29.4% (5/17)	0.0004

*No Bx; The cases which liver biopsy was not performed before treatment.
**RVR indicates rapid virological response, which is defined as HCV RNA PCR-seronegative at week 4 of treatment.

率低下を招いた一因と考えられた．その他にも，今回の我々の対象症例にはセロタイプ2型の症例が比較的多く，統計学的有意差が出なかったことも一因と考えられた．

また，治療前HCV RNA量に関し詳細に検討を行った．今回エントリーされた症例のHCV RNA量は，全体でも平均655 KIU/m*l*であり，HCV RNA量が100 KIU/m*l*以上の，いわゆる「高ウイルス量」症例であっても，1000 KIU/m*l*未満の症例が多かった．このため，HCV RNA量100から1000 KIU/m*l*間に100 KIU/m*l*毎に境界を設定し，各々の境界においてSVR率に有意差があるか検討したところ，セロタイプ1型ではHCV RNA量の境界を400 KIU/m*l*，セロタイプ2型では境界を500 KIU/m*l*まで設定したところSVR率に有意差を認めた（Table 6，セロタイプ1：$p=0.037$，セロタイプ2：$p=0.047$）．このため，セロタイプ1型かつ高ウイルス量の症例であっても，400 KIU/m*l*以下であればSVRを得る可能性が高いことが示唆された．しかしながら，本

Table 5 The rate of SVR according to HCV RNA levels more than 100 KIU/m*l*

Serotype 1	The rate of SVR
HCV RNA < 100 KIU/m*l*	85.7% (6/7)
HCV RNA 100 KIU/m*l* ≦	50.0% (6/12)

Serotype 2	The rate of SVR
HCV RNA < 100 KIU/m*l*	90.5% (19/21)
HCV RNA 100 KIU/m*l* ≦	60.0% (24/40)

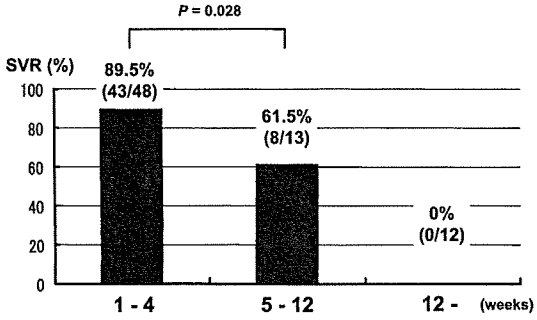

Fig. 3 SVR was observed more frequently in cases with RVR* than in cases with EVR** (p = 0.028). *RVR: Rapid virological response, **EVR: Early virological response

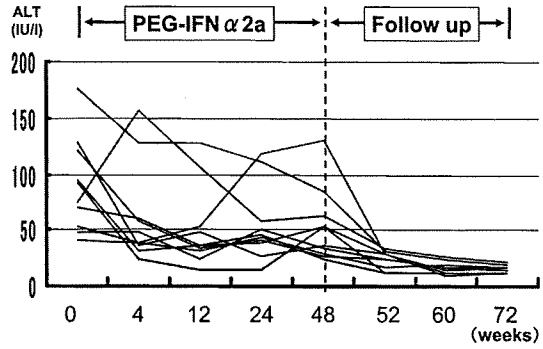

Fig. 4 Serum ALT levels were measured during PEG-IFN monotherapy and follow up period in cases that achieved SVR. In these 10 cases, serum ALT levels increased throughout the administration of PEG-IFN monotherapy, but steadily returned to normal levels during the follow up period. Whereas ALT levels decreased smoothly during both the treatment and follow up periods in 13 cases (Data not shown).

研究ではセロタイプ1型の症例数が少ないため，治療前の至適ウイルス量に関しては，今後も更なる検討が必要であると考えられた．

本研究ではBMIがSVR予測因子とならなかったのは，我々の対象症例では，欧米の報告に比べBMIが正常範囲内（BMI：18.5–25）の患者が多く，いわゆる肥満の症例が少なかったことなどより有意差を認めなかったものと考えられた．

治療前の肝線維化とSVRの検討では，線維化軽度例の方が，有意差をもってSVR率は高値であった．本邦における国内第II相臨床試験の結果では，肝線維化StagingとSVRの割合として，F1 38.9%，F2 39.4%，F3 20.0%であったが，統計学的検討は行われていない[5]．以前我々は，インターフェロン単独療法において，高齢者や肝線維化進行例ではSVR率が低下することを報告したが[12]，PEG-IFN単独療法においても線維化進行例ではSVR率が低いため，線維化軽度例ほど良い治療適応であると考えられた．また，F4ステージの肝硬変例（n=3）では，いずれもSVRは得られず，Zeuzemらの報告と一致した[10]．このため，F4ステージであれば通常の48週投与ではウイルス陰性化の可能性が低いと考えられるため，48週以降の長期投与や治療方針の再考が検討されるべきと考えられた．

IFN治療開始後のSVR予測因子の検討では，早期のウイルス陰性化が重要であることが多数報告されている[11)13)]．本研究においても，治療開始後4週までにHCV RNA定性陰性化が得られたRVR群では，治療開始後5から12週までにHCV RNA定性陰性化が得られたEVR群よりも有意にSVR率が高く（Fig. 3），また多変量解析でも有意差を認めた（Table 4）．以上より，PEG-IFN単独療法の治療効果予測因子として，治療開始4週以内のHCV RNA量定性陰性化（RVR）が重要な因子であることが明らかとなった．また，治療開始5週から12週の間にHCV RNA定性が陰性となったEVR症例では，SVRが得られる可能性がRVRに比べ低いため，投与期間を48週以後も延長することや，可能であれば，低用量のリバビリンを併用する，といった治療法の再考が必要になってくると考えられる．また，12週までにHCV RNA定性が陰性とならなかった症例では，SVRが得られる可能性が極めて低いと考えられるが，このような症例では発癌抑止のためにPEG-IFN治療を長期的に継続するといった選択肢も考えられる[14)15)]．

PEG-IFN単独治療に伴う血清ALT値上昇は少なからず報告されている[6)16)]．また国内第II相臨床試験によ

Table 6 The rate of SVR according to HCV RNA levels in each serotypes

Serotype 1	The rate of SVR	
HCV RNA < 400 KIU/ml	78.5% (11/14)$^{+}$	
HCV RNA 400 KIU/ml ≦	20.0% (1/5)$^{+}$	$^{+}p = 0.037$

Serotype 2	The rate of SVR	
HCV RNA < 500 KIU/ml	79.4% (31/39)$^{++}$	
HCV RNA 500 KIU/ml ≦	54.5% (12/22)$^{++}$	$^{++}p = 0.047$

Table 7 Multivariate logistic regression analysis of the factor associated with achievement of SVR

Factor	Odds ratio (95%CI)	p value
RVR	17.2 (4.38-84.5)	0.0001

Only variables that achieved statistical significance ($p < 0.05$) on multivariate logistic regression are shown.
CI: Confidence Interval

Factor	Sensitivity	Specificity	PPV	NPV
RVR	82.3%	78.7%	89.6%	66.7%

PPV: Positive predictive value, NPV: Negative predictive value

ると，SVR，non-SVRにかかわらずPEG-IFN投与中に血清ALT値上昇を認めたものは180μg投与群で20.7%，90μg投与群で24.6%であった[5]．原因として鉄過剰状態との関連も指摘されているが，はっきりとした機序は不明である[17]．本研究ではSVRを来たした症例のうち，経時的な血清ALT値が追跡可能であった23例について検討したが，そのうち10例（43.4%）でPEG-IFN投与中に血清ALT値の再上昇を来たした．全例SVR例であるため，PEG-IFN投与後に血清ALT値は正常化している．従って，この10例においては，治療中の血清ALT値上昇はPEG-IFNによる副作用であったと考えられ，従来のインターフェロンより血清ALT値上昇率は高いことが明らかになった．PEG-IFN単独療法において治療中に血清ALT値上昇を来たした場合，それが本剤の影響によるものか他の原因によるものかの見極めが重要であると考えられた．

結　語

1，C型慢性肝炎に対するペグインターフェロンα-2a単独48週治療の適応は，若年で肝線維化が軽度の症例である．また，治療前HCV RNA量がセロタイプ1型では400 KIU/ml未満，セロタイプ2型では500 KIU/ml未満の症例が良い適応である．

2，治療開始後のSVR予測因子として，治療開始4週以内のHCV RNA定性陰性が有用である．

3，ペグインターフェロンα-2a単独療法中の血清ALT値の上昇はSVR例の43.4%に認められ，ペグインターフェロンα-2aの副作用と考えられた．

文　献

1) National Institutes of Health Consensus Development Conference Statement. Management of Hepatitis C. Hepatology 2002; 36 (Suppl. 1): S3—S20
2) Strader DB, Wright T, Thomas DL, et al. Diagnosis, management, and treatment of hepatitis C. Hepatology 2004; 39: 1147—1171
3) Perrillo R, Rothstein KD, Rubin R, et al. Comparison of quality of life, work productivity and medical resource utilization of peginterferon alpha 2a vs the combination of interferon alpha 2b plus ribavirin as initial treatment in patients with chronic hepatitis C. J Viral Hepat 2004; 11: 157—165
4) 黒崎雅之，泉　並木．PEG-IFN単独療法．肝胆膵 2006；52：41—46
5) 堺　隆弘，小俣政男，飯野四郎，他．Ro25-8310（ペグインターフェロン　アルファ-2a）のC型慢性肝炎に対する第II相臨床試験．医学と薬学　2003；50：655—672
6) 泉　並木．C型慢性肝炎のインターフェロン治療(3)ペグインターフェロン単独治療はどのような症例で行うのか．臨床消化器内科　2007；22：397—402
7) 泉　並木．C型慢性肝炎の治療 d. ペグインターフェ

ロン療法．臨床医　2004；30：1631—1633
8) Ichida F, Tsuji T, Omata M, et al. New Inuyama classification: new criteria for histological assessment of chronic hepatitis. Inter Hep Comm 1996; 6: 112—119
9) Fried MW, Shiffman ML, Reddy KR, et al. Peginterferon alfa-2a plus ribavirin for chronic hepatitis C virus infection. N Engl J Med 2002; 347: 975—982
10) Zeuzem S, Frinman SV, Rasenack J, et al. Peginterferon alfa-2a in patients with chronic hepatitis C. N Engl J Med 2000; 343: 1666—1672
11) Lee SS, Heathcote EJ, Reddy KR, et al. Prognostic factors and early predictability of sustained viral response with (40KD). J. Hepatorol 2002; 37: 500—506
12) Shakado S, Watanabe H, Sohda T, et al. The sustained virologic response in chronic hepatitis C patients tested with interferon mono-therapy. Med Bull Fukuoka Univ 2006; 33: 31—38
13) Neumann AU, Lam NP, Dahari H, et al. Hepatitis C viral dynamics in vivo and the antiviral efficacy of interferon-α therapy. Science 1998; 282: 103—107
14) Yoshida H, Shiratori Y, Moriyama M, et al. Interferon therapy reduces the risk for hepatocellular carcinoma: national surveillance program of cirrhotic and non-cirrhotic patients with hepatitis C in Japan. IHIT Study Group. Inhibition of Hepatocarcinogenesis by Interferon Therapy. Ann Intern Med 1999; 131: 174—181
15) 建石良介，吉田晴彦，小俣政男．インターフェロンによる発癌予防とその理論的背景．肝胆膵　2006；52：385—391
16) 土井史奈，朝比奈靖浩，泉　並木，他．PEG-IFNα-2a 治療中に ALT の上昇を認めた C 型慢性肝炎の1例．医学と薬学　2006；55：50—52
17) 永島美樹，工藤正俊，鄭　浩柄，他．C 型慢性肝炎に対する PEG-IFNα-2a 製剤投与中の血清 ALT 値上昇についての検討．肝臓　2006；47（Suppl 2）：A416

The prognostic factors of sustained virologic response among patients of chronic hepatitis C treated with peg-interferon alpha 2a monotherapy

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A combination of peginterferon plus ribavirin is recommended therapy for patients with chronic hepatitis C. However, this treatment may influence the occurrence of adverse events induced by ribavirin including anemia or birth defects. This study aimed to reveal the outcome and prognostic factors of peginterferon alpha-2a (PEG-IFN) monotherapy in patients with chronic hepatitis C in Japan. The study included eighty-four patients who were treated with PEG-IFN monotherapy. Fifty-six patients (66.7%) achieved sustained virological response (SVR) in the ITT analysis. When comparing SVR and non-SVR groups, SVR predictable parameters included treatment at a younger age ($p=0.0464$) and early staging of fibrosis ($p=0.0002$). In addition, the most predictable parameter of SVR was serum HCV RNA levels undetectable within 4 weeks after the beginning of the treatment in the multivariate analysis (OR：17.2, 95%CI：4.38–84.5, $p=0.0001$). We suggested that PEG-IFN monotherapy is beneficial for patients who are younger, have mild fibrosis, and lower HCV RNA levels before treatment (<400 KIU/m*l* in serotype 1, <500 KIU/m*l* in serotype 2), and who achieve serum HCV RNA undetectable within 4 weeks from beginning the PEG-IFN monotherapy.

Key words: chronic hepatitis C　PEG-IFN monotherapy　SVR (Sustained virological response)　RVR (Rapid virological response)　liver fibrosis

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Original Article

Early decline of hemoglobin can predict progression of hemolytic anemia during pegylated interferon and ribavirin combination therapy in patients with chronic hepatitis C

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Aim: Ribavirin, used to treat chronic hepatitis C, can induce hemolytic anemia, forcing the discontinuance of treatment. To establish a predictive measure to help circumvent this, we evaluated the relationship of hemoglobin (Hb) decline with the discontinuance of treatment during the progression of ribavirin-induced anemia.

Methods: One hundred and sixteen patients (71% male) with genotype 1 chronic hepatitis C were treated with pegylated interferon (PegIFN) α-2b and ribavirin. The mean age was 50.6 years and 55% were IFN naïve. A decline of Hb concentration by 2 g/dL at two weeks from the start of the treatment ("2 by 2" standard) was adopted as the predictive factor for the progression of anemia.

Results: By applying the "2 by 2" standard, with $\Delta Hb \geq 2$ g/dL (34%, $n = 39$), treatment was discontinued in 12 cases (31%), three of which (8%) because of severe anemia. For $\Delta Hb < 2$ g/dL (64%, $n = 76$), treatment was discontinued in 11 (14%) cases; none due to severe anemia. Ten percent (4/39) of patients showed the minimum $Hb \leq 8.5$ g/dL in the $\Delta Hb \geq 2$ g/dL group, with none in the $\Delta Hb < 2$ g/dL group ($P = 0.001$). Furthermore, the patients with minimum $Hb \leq 8.5$ g/dL were found only in the "2 by 2" standard-positive and low CL/F (<15) group (4/29, 14%).

Conclusion: Monitoring the Hb decline using the "2 by 2" standard can identify patients who are prone to developing severe anemia. Further prospective studies are needed using ribavirin reduction based on the "2 by 2" standard.

Key words: "2 by 2" standard, chronic hepatitis C, pegylated interferon and ribavirin combination therapy, progression of anemia

INTRODUCTION

THE AIM OF antiviral therapy for hepatitis C virus (HCV) is to obtain a sustained viral response (SVR) and to reduce the occurrence rate of hepatocellular carcinoma or hepatic disease-related mortality.[1,2] The current optimal therapy for patients with chronic hepatitis C is a combination of pegylated interferon (PegIFN) and ribavirin. This combination can significantly improve the SVR rate and is recommended as a standard regimen worldwide.[3–8] However, the SVR rates for the combination therapy of ribavirin with PegIFN for naïve patients with HCV genotype 1 has been reported to be 42–52%,[6,9,10] which means that eradication of HCV is not complete in approximately half of these patients. Recently, long-term treatment[11] and a higher dosage

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of drugs[12,13] have been used to try to raise the SVR rate for patients with HCV genotype 1. However, it remains to be established what constitutes satisfactory efficacy. In this study we focused on a treatment strategy to enable the prediction of severe side-effects in order to avoid the need to discontinue treatment and raise the SVR rate by PegIFN and ribavirin combination therapy. It is important that ribavirin, the key drug for eradicating HCV, is continued until the end of treatment in order to attain the maximum SVR rate. Hemolytic anemia induced by ribavirin is known as one of the most important adverse effects in the combination therapy of PegIFN and ribavirin.[14–17] To decrease the discontinuance rate of ribavirin due to severe anemia, epoetin alfa has been used for patients with progressing anemia, which can maintain the dose level of ribavirin as well as the quality of life of the patients.[18–20] However, from a cost-effectiveness standpoint, it would be difficult for this treatment strategy to become standard. Also, side-effects other than anemia arising from an overload of ribavirin mainly due to renal dysfunction cannot be avoided by the additional administration of epoetin alfa.

Hemolysis induced by ribavirin has been suggested to be related to a high plasma concentration of ribavirin.[21] The apparent clearance of ribavirin (CL/F), which reflects its plasma concentration at four weeks after the start of combination therapy, has been used as a predictive factor for ribavirin-induced hemolytic anemia before the start of treatment.[22–24] However, the progression of hemolytic anemia occurs due not only to hemolysis, but also impaired hematogenous function. On the other hand, hemoglobin (Hb) dynamics directly reflect the degree of progression of anemia. We have reported that the early decline of Hb correlates with the progression of anemia during IFN and ribavirin combination therapy.[25] It is necessary to verify that a similar early predictor for the progression of anemia can be adopted in PegIFN and ribavirin combination therapy, since PegIFN is known to induce less depression of bone marrow function than usual IFN.

In this study, we evaluated the utility of the early decline of Hb in comparison with the CL/F to predict the progression of anemia in the combination therapy of PegIFN and ribavirin.

METHODS

Patients

THIS STUDY WAS conducted at 12 institutions in Japan. A total of 116 patients with chronic hepatitis C were enrolled and treated with a combination of PegIFN and ribavirin. All patients were anti-hepatitis C virus antibody positive, had HCV-RNA detectable in their serum by the polymerase chain reaction (PCR) method, and showed elevated serum alanine transaminase (ALT) (above the upper limit of the normal), serum Hb concentration ≥12 g/dL, neutrocytes ≥1500/mm^3 and platelets ≥10^5/mm^3 within six months before the treatment. Exclusion criteria were the presence of hepatitis B surface antigen, antihuman immunodeficiency virus antibody and other forms of liver disease (alcoholic liver disease, hepatotoxic drugs, autoimmune hepatitis).

Table 1 Patient characteristics

Age (years)	50.6 ± 10.1 (24–70)
Gender (male/female)	82/34 (male 70.7%)
Body weight (kg)	64.5 ± 11.1
Previous IFN therapy (naïve/relapser/no responder)	64/38/14
HCV-RNA level (KIU/L) (<500/500–850/850<)	18/27/71
ALT (IU/L)	110 ± 60 (33–76)
Crnn (mg/dL)	0.9 ± 0.2
Liver histology	
Fibrosis (F1/F2/F3/unknown)	35/49/31/1
Activity (A1/A2/A3/A4)	15/33/56/12
WBC (/mm^3)	5317 ± 1207
Neutrocytes (/mm^3)	2778 ± 902
Platelets (×10^4/mm^3)	17.4 ± 4.0
RBC (×10^4/mm^3)	459 ± 41
Hemoglobin (g/dL)	14.5 ± 1.2

Data are given as the mean ± SD.
ALT, alanine transaminase; RBC, red blood cells; WBC, white blood cells.

The baseline characteristics of the patients are shown in Table 1. The mean age was 50.6 ± 10.1 years, and 71% (82 patients) were male. All patients had HCV-RNA with genotype 1 and high viral loads (more than 10^5 copies/mL serum by Amplicor-HCV monitor assay). The mean ALT level was 110 ± 60 IU/L. Sixty-four patients (55%) were IFN naïve and the others were undergoing retreatment.

Treatment schedule

All patients were treated with a combination of PegIFN α-2b (Pegintron; Schering-Plough, Kenilworth, NJ, USA) and ribavirin (Rebetol; Schering-Plough) for 48 weeks. PegIFN was administered at a mean of 1.5 μg/kg body weight subcutaneously once a week. Ribavirin was given orally twice a day for the total dose. Dosages of both medications were decided based on the

body weight of the patients: those with a body weight of 40–60 kilograms (kg) were given PegIFN 75 μg/body and ribavirin 600 mg/day, those with a body weight of 60–80 kg were given PegIFN 105 μg/body and ribavirin 800 mg/day, and those with a body weight of 80–100 kg were given PegIFN 135 μg/body and ribavirin 1000 mg/day. The PegIFN dose was reduced by 50% if the neutrocyte count was below 750/mm^3 or the platelet (Plt) count was below 8×10^4/mm^3. The PegIFN was discontinued if the neutrocyte count was below 500/mm^3 or the Plt count was below 5.0×10^4/mm^3. The ribavirin dose of 200 mg was reduced when the Hb concentration decreased to less than 10 g/dL and the ribavirin was discontinued when the Hb concentration decreased to less than 8.5 g/dL, in accordance with the drug information for ribavirin. No ferric medicine or erythropoietin to prevent anemia was administered.

Patients with persistently undetectable HCV-RNA six-months after the end of treatment were considered to have achieved SVR.

Blood tests

All patients were examined for serum HCV-RNA level, hematological and biochemical tests just before therapy, at the end of week 2 and every four weeks during the treatment. When the treatment was completed, the patients were assessed every four weeks up to 24 weeks after the end of treatment.

Total ribavirin clearance

Using the method of Kamar *et al.*, CL/F at the start of the treatment was calculated as follows: CL/F (L/h) = $32.3 \times BW \times (1 - 0.0094 \times age) \times (1 - 0.42 \times sex)/Scr$ (BW, body weight; sex = 0 for male and 1 for female; Scr = serum creatinine).[17]

Definition of "severe anemia" leading to the discontinuance of ribavirin

In this study, the "discontinuance of ribavirin due to severe anemia" was defined as follows: discontinuance of ribavirin due to a decrease of Hb to less than 8.5 g/dL or clinical symptoms of anemia associated with a decrease of Hb of more than 3 g/dL from the start of the combination therapy.

Statistical analysis

Age, body weight, ribavirin dosage/body weight, white blood cell count, red blood cell count, Hb concentration, Plt, serum ALT levels and serum creatinine are expressed as mean ± SD. The SVR rate was evaluated using the intention-to-treat analysis (ITT analysis). The differences in proportions were tested by the χ^2-test and Mantel-Haenszel χ^2-test. A value of $P < 0.05$ (two-tailed) was considered to indicate significance. All calculations were performed by SAS program 9.1 (SAS Institute, Cary, NC, USA).

RESULTS

Frequency and reasons for dose reduction or discontinuance of PegIFN and/or ribavirin

OF THE 116 patients, 92 completed 48 weeks of therapy, but 24 patients (21%) had to discontinue both PegIFN and ribavirin. Thirty-nine patients (34%) completed the entire treatment schedule without reduction or discontinuance of either drug. The ribavirin dose was decreased for 39 patients (34%) and the PegIFN dose was decreased for 33 patients (28%), including 19 patients for whom both drugs had to be reduced. The reasons for discontinuance of both drugs included anemia, thyroid dysfunction, skin eruption and neutropenia, with the major reasons being anemia (17%) and thyroid dysfunction (17%).

Efficacy of the combination therapy with dose reduction or discontinuance of PegIFN and/or ribavirin

The SVR rate was 57% (66/116) for all according to ITT analysis. According to the category of response to previous IFN therapy, the SVR rates were 43% (6/14) in

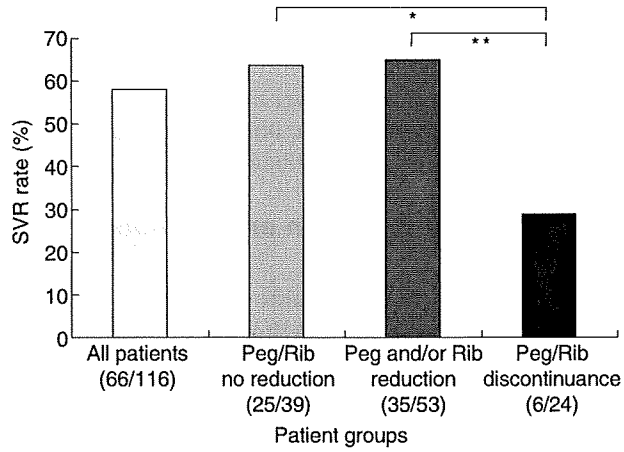

Figure 1 SVR rate due to PegIFN/ribavirin dose reduction or discontinuance. (□), All patients; (▨), patients without dose reduction; (▩), patients with dose reduction; (■), patients with drug discontinuance. Significant levels: $*P = 0.003$; $**P = 0.001$.