Insulin resistance substudy: prevalence of insulin resistance in non-diabetic patients

Patient characteristics

The characteristics of the patients enrolled in the IR substudy are shown in Table 1. Age distribution, proportion of male gender and proportion of clinical stage in the HBV+chronic, HCV+chronic and HCV+cleared groups were similar in the diabetes study and the IR substudy (Table 1). Hypertension was most prevalent in the HCV+chronic group, hyperlipidaemia was less prevalent in the HCV+chronic group owing to a lower total cholesterol level, and obesity was most prevalent in the HCV+cleared group.

Table 3. Clinical factors associated with development of diabetes mellitus in HBV+ or HCV+chronic patients using multivariate logistic regression analysis in diabetes study

Variables	Odds ratio	95% confidence interval	*P*-value
Male (vs female)	2.404	1.497–3.861	0.0003
Age (vs ≤ 49)			
50–59	4.484	2.119–9.434	< 0.0001
≥60	3.774	1.792–7.937	0.0005
Clinical stage (vs asymptomatic carrier)			
Chronic hepatitis	1.408	0.698–2.841	0.3393
Cirrhosis	2.273	1.048–4.926	0.0376
HCV (vs HBV)	1.669	0.917–3.040	0.0936

HBV, hepatitis B virus; HCV, hepatitis C virus.

Prevalence of insulin resistance with various clinical backgrounds

The prevalence of IR was 49/135 (36.3%) in the HBV+chronic group, 126/232 (54.3%) in the HCV+chronic group and 20/56 (35.7%) in the HCV+cleared group, with the prevalence in the HCV+chronic group being significantly higher than that in the HBV+chronic group ($P < 0.005$) (Table 4). This result also applied when considering males and females separately. According to stratified age, the prevalence of IR was higher above the age of 50 years in the HCV+chronic group compared with the HBV+chronic group. According to clinical stage, IR in chronic hepatitis was more prevalent in the HCV+chronic group than in the HBV+chronic group ($P < 0.05$), but the prevalence was similar in asymptomatic carrier patients in these two groups (Table 4).

Table 4. Prevalence of insulin resistance with various clinical backgrounds in insulin resistance substudy

				P-value		
	HBV+ chronic	HCV+ chronic	HCV+ cleared	HBV+chronic vs HCV+chronic	HCV+chronic vs HCV+cleared	HBV+chronic vs HCV+cleared
Number	135	232	56			
HOMA-IR≥2.0	49 (36.3%)	126 (54.3%)	20 (35.7%)	< 0.005	< 0.05	NS
Gender						
Male	30 (39.0%)	51 (54.8%)	15 (39.5%)	< 0.05	NS	NS
Female	19 (32.8%)	75 (54.0%)	5 (27.8%)	< 0.01	< 0.05	NS
Age						
≤ 49	34 (43.0%)	18 (40.9%)	6 (30.0%)	NS	NS	NS
50–59	10 (25.6%)	30 (57.7%)	4 (26.7%)	< 0.005	< 0.05	NS
≥60	5 (29.4%)	78 (57.4%)	10 (47.6%)	< 0.05	NS	NS
Clinical stage						
ASC	10 (25.0%)	12 (32.4%)	–	NS		
CH	32 (41.6%)	80 (57.1%)	–	< 0.05		
LC	7 (38.9%)	34 (61.8%)	–	NS		
Hypertension						
(+)	0 (0%)	32 (65.3%)	2 (40%)	< 0.01	NS	NS
(−)	49 (38.0%)	94 (51.4%)	18 (36.0%)	< 0.05	< 0.05	NS
Hyperlipidaemia						
(+)	18 (56.3%)	15 (45.5%)	5 (29.4%)	NS	NS	NS
(−)	31 (30.4%)	111 (55.8%)	15 (38.5%)	< 0.0001	< 0.05	NS
Obesity						
(+)	15 (78.9%)	34 (85.0%)	10 (50%)	NS	*P*< 0.01	NS
(−)	25 (27.8%)	81 (47.9%)	8 (26.7%)	*P*< 0.005	*P*< 0.05	NS

ASC, asymptomatic carrier; CH, chronic hepatitis; HBV, hepatitis B virus; HCV, hepatitis C virus; HOMA-IR, homeostasis model of insulin resistance; LC, cirrhosis; NS, not significant.

Table 5. Clinical factors associated with insulin resistance in HBV+chronic or HCV+chronic patients using multivariate logistic regression analysis in insulin resistance substudy

Variables	Odds ratio	95% confidence interval	*P*-value
Age (vs ≤ 49)			
50–59	1.133	0.534–2.404	0.7444
≥60	1.642	0.769–3.509	0.2003
Body mass index (vs < 25)			
≥25	5.765	2.563–12.967	< 0.001
Clinical stage (vs asymptomatic carrier)			
Chronic hepatitis	1.764	0.792–3.922	0.1652
Cirrhosis	2.183	0.820–5.814	0.1180
HCV (vs HBV)	1.531	0.781–3.003	0.2154
AST≥40 (vs < 40)	0.980	0.475–2.021	0.9567
ALT≥40 (vs < 40)	2.595	1.279–5.265	0.0082
γ-glutamyl transpeptidase (vs < 40 IU/L)			
≥40	2.100	1.108–3.981	0.0229
Triglyceride (vs < 100 mg/dl)			
≥100	1.966	1.077–3.588	0.0276

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HBV, hepatitis B virus; HCV, hepatitis C virus.

Clinical factors associated with insulin resistance in the HBV+chronic and HCV+chronic groups

One hundred and seventy-five of 367 patients had a HOMA-IR >2.0, were older (55.9 ± 14.1 vs 52.4 ± 15.6, $P < 0.05$), had a higher proportion of HCV infection (72.0 vs 55.2%, $P < 0.01$), had more advanced liver disease (asymptomatic carrier/chronic hepatitis/cirrhosis; 22/112/41 vs 55/105/32, $P < 0.001$) and had higher BMI (24.2 ± 3.0 vs 21.4 ± 2.7, $P < 0.0001$), fasting plasma glucose (101 ± 10 vs 96 ± 8, $P < 0.0001$), immunoreactive insulin (13.3 ± 5.2 vs 5.5 ± 1.7, $P < 0.0001$), AST (56 ± 58 vs 37 ± 23, $P < 0.0001$), ALT (66 ± 107 vs 36 ± 30, $P < 0.0001$), γ-glutamyl transpeptidase (50 ± 69 vs 30 ± 32, $P < 0.0001$) and triglyceride levels (99 ± 46 vs 84 ± 54, $P < 0.0001$) than those with a HOMA-IR < 2.0.

Multivariate logistic regression analysis showed BMI ≥25, ALT ≥40, γ-glutamyl transpeptidase ≥40 and triglyceride ≥100 as independent risk factors, with ORs of 5.765, 2.595, 2.100 and 1.966, respectively, but HCV infection compared with HBV infection was not found to be a statistically significant variable (Table 7).

Clinical factors associated with insulin resistance in the HCV+chronic and HCV+cleared groups

One hundred and forty-six patients showed a HOMA-IR >2.0 among 288 patients in the HCV+chronic and HCV+cleared groups. They were older (60.3 ± 11.3 vs 56.5 ± 14.5, $P < 0.05$), had a higher proportion of HCV infection (86.3 vs 74.6%, $P < 0.05$) and had a higher BMI (24.1 ± 2.9 vs 21.8 ± 3.0, $P < < 0.0001$), fasting plasma glucose (102 ± 11 vs 97 ± 8, $P < 0.0001$), immunoreactive insulin (12.9 ± 4.9 vs 5.4 ± 1.7, $P < 0.0001$), AST (49 ± 27 vs 38 ± 24, $P < 0.0005$), ALT (51 ± 35 vs 35 ± 29, $P < 0.0001$) and γ-glutamyl transpeptidase levels (43 ± 43 vs 34 ± 38, $P < 0.05$) than those with a HOMA-IR < 2.0.

Multivariate logistic regression analysis showed age over 60, BMI ≥25 and ALT ≥40 as independent risk factors for IR, with ORs of 2.392, 4.749 and 4.634 respectively. However, ongoing HCV infection compared with HCV eradication was not found to be a statistically significant variable (Table 6).

Clinical factors associated with insulin resistance in asymptomatic carrier patients in the HCV+chronic and HCV+cleared groups

Thirteen variables, including HOMA-IR were compared between 37 asymptomatic carrier patients with HCV infection and 56 patients whose HCV had cleared after interferon treatment. Asymptomatic carriers with HCV infection showed a smaller proportion of male gender (27 vs 68%, $P < 0.0005$), a higher level of ALT (23 ± 9 vs 19 ± 9, $P < 0.05$) and a greater proportion of HCV genotype 1 (80 vs 47.8%, $P < 0.05$) compared with patients whose HCV infection had cleared, but the proportion of patients with a HOMA-IR above 2.0 was similar in the two groups (32 vs 36%, $P = 0.8$). These results suggest that HCV infection alone might not be associated with IR.

Discussion

This cross-sectional study showed a weak and not statistically significant higher association of diabetes with HCV infection compared with HBV infection, and advanced liver disease such as cirrhosis and other traditional risk factors for diabetes such as older age and male gender were more closely involved than HCV infection. This result also held true for the association between HCV infection and IR in patients without overt diabetes.

The prevalence of DM increased according to the progression of liver disease in patients with either HBV or HCV infection, and it was higher in patients with HCV than in those with HBV in every clinical stage except for asymptomatic carrier (Table 2). The prevalence of DM was similar in asymptomatic carrier patients with HCV and those with HBV (6.4 and 6.0%, $P = 0.67$) and, furthermore, it was also similar in

Liver International (2008)

asymptomatic carrier patients with HCV and those whose HCV had cleared (6.4 and 9.0%, $P=0.60$), with both groups showing almost normal ALT levels. These observations suggest that not HCV infection itself but the resultant ongoing inflammation and fibrosis of the liver might determine a higher risk for DM. This was also applicable to IR in patients without overt DM, as the present study showed that liver enzyme abnormalities such as elevation of ALT, γ-glutamyl transpeptidase and BMI over 25.0 were independent risk factors for IR but HCV infection itself was not. These results are discordant with the recent report of a transgenic mouse model, in which the HCV core protein was shown to contribute directly to the development of IR by disturbing tyrosine phosphorylation of insulin receptor substrate 1 without inflammation and fibrosis during the observation period (29).

Treatment with interferon or interferon plus ribavirin is now a standard regimen for hepatitis C, and IR was reported to be improved in patients whose HCV had cleared and was not affected in those with relapse or no response after interferon treatment (30). In the present study, we found the prevalence of IR to be higher in patients infected with HCV than in those whose HCV had been cleared, but multivariate logistic regression analysis did not extract HCV infection as an independent risk factor for IR after adjusting for age, BMI and ALT (Table 6). Furthermore, we found no difference in the prevalence of IR between HCV-infected asymptomatic carrier patients and patients whose HCV had cleared (32 vs 36%, $P=0.83$), both with normal ALT levels.

Table 6. Clinical factors associated with insulin resistance in HCV+chronic or HCV+cleared patients using multivariate logistic regression analysis in insulin resistance substudy

Variables	Odds ratio	95% confidence interval	*P*-value
Male (vs female)	0.870	0.473–1.603	0.6550
Age (vs ≤ 49)			
50–59	1.715	0.700–4.202	0.2376
≥60	2.392	1.093–5.236	0.0290
Body mass index (vs < 25)			
≥25	4.749	2.170–10.393	< 0.0001
HCV-RNA (+) [vs (−)]	1.518	0.646–3.570	0.3383
AST ≥40 (vs < 40 IU/L)	1.101	0.502–2.417	0.8090
ALT ≥40 (vs < 40 IU/L))	4.634	2.153–9.973	< 0.0001
Platelet (vs ≥200 × 10^9/L)			
< 200	1.155	0.602–2.213	0.6647
Total cholesterol (vs ≥180 mg/dl)			
< 180	1.042	0.564–1.925	0.8946

ALT, alanine aminotransferase; AST, aspartate aminotransferase; HCV, hepatitis C virus; HCV-RNA, hepatitis C virus ribonucleic acid.

There are six major genotypes of HCV classified in the world (31). Among them, genotype 3 has been reported to be closely associated with steatosis in the liver (32), but genotype 3 is not common in Japan. There have been several reports on the association between genotype 1 or 2 and DM (13, 16, 21). In the present study, we could not confirm these findings because there were no differences in the prevalence of DM or IR between patients with genotypes 1 and 2.

The diabetes study is a retrospective one, and hence some important confounding risk factors for diabetes such as BMI were defective. However, similar results were obtained in the prospective IR substudy on the prevalence of IR, antecedent to the development of diabetes, after adjustment for additional confounding risk factors. In the diabetes study, BMI data were available in 396 (48%) of 830 patients and the prevalence of diabetes in these patients was higher in those with HCV infection than in those with HBV infection (33/266 (12.4%) vs 7/130 (5.4%), $P=0.033$). Multivariate logistic regression analysis showed that the OR of HCV infection was 2.07 after adjustment for age, gender, BMI and clinical stage without statistical significance ($P=0.1347$).

Previous studies by Caronia *et al.* (15) and Mason *et al.* (21) demonstrated a higher prevalence of DM in patients with HCV-related disease than in those with HBV-related disease in agreement with our findings. Although the present study failed to ascertain HCV infection as an independent factor for diabetes by multivariate logistic regression analysis in discordance with the previous studies (15, 21), the relative OR for diabetes was 1.67 times higher in patients with HCV than in those with HBV. Considering that there is no normal control group, we could not deny the association of HCV infection with DM. Furthermore, HBV infections occur vertically from their mothers at birth in most Japanese patients with chronic hepatitis B, indicating that the duration of infection is almost the same as the age of the patients, but in this study of community-acquired HCV the duration of infection could not be estimated and it is difficult to establish the temporality of the development of hepatitis and diabetes.

In conclusion, this study showed a higher prevalence of DM and IR in patients with HCV infection than in those with HBV infection. However, other factors such as age, male gender, BMI and cirrhosis seemed to be more important risk factors for the development of glucose abnormalities in Japan.

References

1. Bruix J, Calvet X, Costa J, *et al.* Prevalence of antibodies to hepatitis C virus in Spanish patients with hepatocellular carcinoma and hepatic cirrhosis. *Lancet* 1989; **2**: 1004–6.
2. Tong MJ, El-Farra NS, Reikes AR, Co RL. Clinical outcome after transfusion-associated hepatitis C. *N Engl J Med* 1995; **332**: 1463–6.
3. Alter MJ, Margolis HS, Krawczynski K, *et al.* The natural history of community-acquired hepatitis C in the United States. The sentinel counties chronic non-A, non-B hepatitis study team. *N Engl J Med* 1992; **327**: 1899–1905.
4. Yazaki Y, Kadowaki T. Combating diabetes and obesity in Japan. *Nat Med* 2006; **12**: 73–4.
5. Yach D, Stuckler D, Brownell KD. Epidemiologic and economic consequences of the global epidemics of obesity and diabetes. *Nat Med* 2006; **12**: 62–6.
6. Tuomilehto J, Lindstrom J, Eriksson JG, *et al.* Finnish diabetes prevention study group. Prevention of type 2 diabetes mellitus by changes in lifestyle among subjects with impaired glucose tolerance. *N Engl J Med* 2001; **344**: 1343–50.
7. DeFronzo RA, Simonson D, Ferranninii E. Hepatic and peripheral insulin resistance: a common feature of type 2 (non-insulin dependent) and type 1 (insulin-dependent) diabetes mellitus. *Diabetologia* 1982; **23**: 313–9.
8. Kingston ME, Ali MA, Atiyeh M, Donnelly RJ. Diabetes mellitus in chronic active hepatitis and cirrhosis. *Gastroenterology* 1984; **87**: 688–94.
9. Petrides AS. Liver disease and diabetes mellitus. *Diabetes Rev* 1994; **2**: 2–18.
10. Petrides AS, Vogt C, Schulze-Berge D, Matthews D, Strohmeyer G. Pathogenesis of glucose intolerance and diabetes mellitus in cirrhosis. *Hepatology* 1994; **19**: 616–27.
11. El-Serag HB, Tran Thomas, Everhart JE. Diabetes increases the risk of chronic liver disease and hepatocellular carcinoma. *Gastroenterology* 2004; **126**: 460–8.
12. Matteoni C, Younossi Z, Gramlich T, Bopari N, Liu Y, McCullough A. Nonalcoholic fatty liver disease: a spectrum of clinical and pathological severity. *Gastroenterology* 1999; **116**: 1413–9.
13. Allison ME, Wreghitt T, Palmer CR, Alexander GJ. Evidence for a link between hepatitis C virus infection and diabetes mellitus in a cirrhotic population. *J Hepatol* 1994; **21**: 1135–9.
14. Grimbert S, Valensi P, Levy-Marchal C, *et al.* High prevalence of diabetes mellitus in patients with chronic hepatitis C. A case-control study. *Gastroenterol Clin Biol* 1996; **20**: 544–8.
15. Caronia S, Taylor K, Pagliaro L, *et al.* Further evidence for an association between non-insulin-dependent diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **30**: 1059–63.
16. Knobler H, Schihmanter R, Zifroni A, Fenakel G, Schattner A. Increased risk of type 2 diabetes in noncirrhotic patients with chronic hepatitis C virus infection. *Mayo Clin Proc* 2000; **75**: 355–9.
17. Mehta SH, Brancati FL, Sulkowski MS, Strathdee SA, Szklo M, Thomas DL. Prevalence of type 2 diabetes mellitus among persons with hepatitis C virus infection in the United States. *Ann Intern Med* 2000; **133**: 592–9.
18. Lecube A, Hernandez C, Genesca J, Esteban JI, Jardi R, Simo R. High prevalence of glucose abnormalities in patients with hepatitis C virus infection: a multivariate analysis considering the liver injury. *Diabetes Care* 2004; **27**: 1171–5.
19. Gray H, Wreghitt T, Stratton IM, Alexander GJ, Turner RC, O'Rahilly S. High prevalence of hepatitis C infection in Afro-Caribbean patients with type 2 diabetes and abnormal liver function tests. *Diabet Med* 1995; **12**: 244–9.
20. Simo R, Hernandez C, Genesca J, Jardi R, Mesa J. High prevalence of hepatitis C virus infection in diabetic patients. *Diabetes Care* 1996; **19**: 998–1000.
21. Mason AL, Lau JY, Hoang N, *et al.* Association of diabetes mellitus and chronic hepatitis C virus infection. *Hepatology* 1999; **29**: 328–33.
22. Mangia A, Schiavone G, Lezzi G, *et al.* HCV and diabetes mellitus: evidence for a negative association. *Am J Gastroenterol* 1998; **93**: 2363–7.
23. Zein NN, Abdulkarim AS, Wiesner RH, Egan KS, Persing DH. Prevalence of diabetes mellitus in patients with end-stage liver cirrhosis due to hepatitis C, alcohol, or cholestatic disease. *J Hepatol* 2000; **32**: 209–17.
24. Arao M, Murase K, Kusakabe A, *et al.* Prevalence of diabetes mellitus in Japanese patients infected chronically with hepatitis C virus. *J Gastroenterol* 2003; **38**: 355–60.
25. Gaiani S, Gramantieri L, Venturoli N, *et al.* What is the criterion for differentiating chronic hepatitis from compensated cirrhosis? A prospective study comparing ultrasonography and percutaneous liver biopsy. *J Hepatol* 1997; **27**: 979–85.
26. Matthws DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. Homeostasis model assessment; insulin resistance and β-cell function from fasting plasma glucose and insulin concentrations in man. *Diabetologia* 1985; **28**: 412–9.
27. Young KKY, Resnick RM, Meyers TW. Detection of hepatitis C virus RNA by a combined reverse transcription-polymerase chain reaction assay. *J Clin Microbiol* 1993; **31**: 882–6.
28. Tanaka T, Tsukiyama-Kohara K, Yamaguchi K, *et al.* Significance of specific antibody assay for genotyping of hepatitis C virus. *Hepatology* 1994; **19**: 1347–53.
29. Shintani Y, Fujie H, Miyoshi H, *et al.* Hepatitis C virus infection and diabetes: direct involvement of the virus in the development of insulin resistance. *Gastroenterology* 2004; **126**: 840–8.
30. Romero-Gomez M, Del Mar Viloria M, Andrade RJ, *et al.* Insulin resistance impairs sustained response rate to peginterferon plus ribavirin in chronic hepatitis C patients. *Gastroenterology* 2005; **128**: 636–41.
31. Simmonds P, Holmes EC, Cha TA, *et al.* Classification of hepatitis C virus into six major genotypes and a series of subtypes by phylogenetic analysis of the NS-5 region. *J Gen Virol* 1993; **74**: 2391–9.
32. Rubbia-Brandt L, Quadri R, Abid K, *et al.* Hepatocyte steatosis is a cytopathic effect of hepatitis C virus genotype 3. *J Hepatol* 2000; **33**: 106–15.

Liver International (2008)

J Gastroenterol 2008; 43:711–719
DOI 10.1007/s00535-008-2222-5

Journal of Gastroenterology

Efficacy of combination therapy of antiviral and immunosuppressive drugs for the treatment of severe acute exacerbation of chronic hepatitis B

Keiichi Fujiwara[1], Shin Yasui[1], Yutaka Yonemitsu[1], Kenichi Fukai[1], Makoto Arai[1], Fumio Imazeki[1], Akihiro Suzuki[2], Hiroshi Suzuki[2], Tomohito Sadahiro[3], Shigeto Oda[3], and Osamu Yokosuka[1]

[1] Department of Medicine and Clinical Oncology, Graduate School of Medicine, Chiba University, 1-8-1 Inohana, Chuo-ku, Chiba 260-8670, Japan
[2] Takushinkai Tatsumi Hospital, Ichihara, Japan
[3] Department of Emergency and Critical Care Medicine, Graduate School of Medicine, Chiba University, Chiba, Japan

Background. Patients with severe exacerbation of chronic hepatitis B, sometimes developing into fulminant liver failure, are at high risk for mortality even with antiviral therapy. The efficacy of immunosuppressive therapy in clinically severe exacerbation of chronic hepatitis B has not been well demonstrated. In this study, we evaluated the efficacy of the early introduction of immunosuppressive therapy in combination with antiviral therapy in such patients. ***Methods.*** Forty-two patients, 29 men and 13 women, were defined as having severe exacerbation of chronic hepatitis B based on our uniform criteria, and were enrolled in this study. Sixteen patients between 1982 and 1996 were analyzed retrospectively. We defined the criteria of severe disease in 1997, and then began to introduce sufficient doses of corticosteroids prospectively. Nucleoside analogs were administered in combination with corticosteroids after 1999. Twenty-six patients between 1997 and 2007 were analyzed prospectively. ***Results.*** In the retrospective study between 1982 and 1996, four of 16 (25%) patients recovered. In the prospective study between 1997 and 2007, 17 of 26 (65%) patients recovered; 15 of 17 patients treated with corticosteroids with or without antiviral drugs within 10 days after the diagnosis of severe disease recovered, none of five treated similarly but later than 10 days after the diagnosis recovered, and two of three treated with antiviral drugs recovered. ***Conclusions.*** The early introduction of sufficient doses of corticosteroids and nucleoside analogs could be one option for reversing the potential deterioration of patients with clinically severe, life-threatening exacerbation of chronic hepatitis B.

Key words: chronic hepatitis B, severe exacerbation, immunosuppressive therapy, antiviral therapy

Received: March 1, 2008 / Accepted: May 17, 2008
Reprint requests to: K. Fujiwara

Introduction

It is well recognized that exacerbation of hepatitis B may occur in chronic hepatitis B virus (HBV) carriers, spontaneously or in relation to cytotoxic or immunosuppressive therapy. A clinical picture of acute hepatitis, and even severe exacerbation, and sometimes fulminant liver failure, may develop that is associated with high mortality.[1] In a retrospective survey in Japan, a 53% incidence of severe hepatitis with a 24% mortality rate (mortality rate of 45% in severe hepatitis) has been reported in relation to chemotherapy in HBV carriers with hematologic malignancies.[2] For the treatment of patients with severe exacerbation without malignancies who progress to serious deterioration, liver transplantation may be a viable option. However, the problems of a shortage of donor livers in Japan and the high cost of the procedure still remain. Thus, therapies other than transplantation must be further investigated for chronic hepatitis B patients with severe exacerbation.

In HBV infection, liver injury is considered to be induced mainly by cytotoxic T lymphocyte-mediated cytolytic pathways of infected hepatocytes.[3] Sjogren et al.[4] suggested that corticosteroids modulate the activity of chronic hepatitis B by suppression of the host immune response to hepatitis B virus antigens, based on a comparison of alanine aminotransferase (ALT) and IgM anti-hepatitis B core antibody (IgM-HBc) levels during the course of short-term high-dose prednisolone therapy. Accordingly, treating chronic hepatitis B patients with corticosteroids to inhibit excessive immune response and prevent cytolysis of infected hepatocytes is a reasonable treatment decision.

Corticosteroids have been administered to treat active chronic hepatitis B since the 1970s. However, in recent years, because the advantage of their use has not been confirmed by controlled studies, their use for the routine management of chronic hepatitis B has fallen

out of favor.[5-7] However, these previous studies dealt mainly with cases of clinically nonsevere hepatitis that was not urgently life threatening, and the effects of corticosteroid treatment for severe, potentially life-threatening exacerbation of chronic hepatitis B, as well as the timing and dose of such treatment, have not been well demonstrated. Lau et al.[8] reported that the reintroduction of long-term, high-dose corticosteroids in the early phase of reactivation after withdrawal of immunosuppressive therapy prevents both progressive clinical deterioration and potentially the need for orthotopic liver transplantation.

In our previous study, we investigated the clinicopathological features of patients with severe exacerbation selected by uniform criteria and treated by the early introduction or reintroduction of sufficient doses of corticosteroids, and reported that the introduction of high-dose corticosteroids can significantly reverse deterioration in patients with clinically severe, life-threatening exacerbation of chronic hepatitis B, compared with historical controls, when used in the early stage of illness.[9] Recently, nucleoside analogs have been administered safely even to patients with severe disease,[10-15] but their benefits in terms of the clinical outcome have still to be clarified.

In this study, we treated patients with severe disease by early initiation of sufficient doses of corticosteroids and nucleoside analogs prospectively to clarify the benefits and limitations of the therapy for amelioration of clinically severe exacerbation of chronic hepatitis B.

Patients and methods

Patients

Forty-two patients with severe exacerbation of chronic hepatitis B who were admitted to our liver unit (Chiba University Hospital and related hospitals) between 1982 and 2007 were studied. The diagnosis of chronic hepatitis B viral carrier was made based on either hepatitis B surface antigen (HBsAg) positivity for at least 6 months before entry, or, in patients with a follow-up period of less than 6 months before entry, on hepatitis B surface antigen positivity, a high anti-hepatitis B core antibody (HBcAb) titer, and IgM-HBc negativity or a low titer. The patients fulfilling all of the following three criteria during the disease course were defined as having severe exacerbation: prothrombin time (PT) activity less than 60% of normal control, total bilirubin (T-Bil) greater than 3.0 mg/dl, and ALT greater than 300 IU/l. All patients were in poor general condition, including general malaise, fatigue, jaundice, edema, ascites, and encephalopathy. A histological examination was performed in patients during the convalescent phase or after their death.

All patients were negative for IgM anti-hepatitis A virus (HAV) antibody, anti-hepatitis D antibody, anti-hepatitis C virus (HCV) antibody, HCV RNA, IgM anti-Epstein-Barr virus antibody (IgM-EBV), IgM anti-herpes simplex virus antibody (IgM-HSV), IgM anti-cytomegalovirus antibody (IgM-CMV), anti-nuclear antibody, anti-smooth muscle antibody, liver kidney microsomal antibody 1, and anti-mitochondrial antibody. Patients with histories of recent exposure to drugs and chemical agents as well as of recent heavy alcohol intake were excluded. One patient was human immunodeficiency virus (HIV) positive, but had no clinical evidence of acquired immune deficiency syndrome.

Protocols for treatment

Twenty-six patients treated after 1997 were examined prospectively and 16 treated before 1996 were examined retrospectively. Of the prospectively studied group, informed consent was obtained from the patients or appropriate family members. Patients were treated by early introduction of corticosteroids (CS) (early CS) as follows: 60 mg or more of prednisolone daily was administered within 10 days after the diagnosis of severe disease using the above-mentioned criteria. This dose was maintained for a minimum of 4 days. When the patient showed a trend toward remission in PT, the dose was reduced by 10 mg at least every 4 days to 30 mg. Then, the dose was further tapered by 2.5 or 5 mg every 2 weeks or longer, depending on the decreasing trend of the ALT level, in the period of immunosuppressive monotherapy before nucleoside analogs were administered. Afterward, lamivudine (LMV), adefovir (ADV), and entecavir (ETV), nucleoside analogs with significant inhibition of HBV DNA polymerase, could be used safely in patients with severe disease.[10-15] In Japan, LMV for HIV and chronic hepatitis B became available in 1997 and 2000, respectively. The CS dose was reduced more rapidly and tapered off while monitoring the viral load reduction after 1999, when we began to administer nucleoside analogs.

Patients who had already passed more than 10 days after the diagnosis before being admitted to our unit were treated by the delayed introduction of corticosteroids (delayed CS). Patients with marked prolongation of PT were treated with 1000 mg of methylprednisolone daily for 3 days followed by the same prednisolone therapy as described above. After 1998, LMV was administered at a daily dose of 100–300 mg. ADV was administered at a daily dose of 10 mg to LMV-breakthrough hepatitis. ETV was administered at a daily dose of 0.5–1.0 mg. Patients who showed a trend toward remission or irreversible hepatic failure at admission

were treated with intravenous glycyrrhizin (stronger neominophagen C, SNMC), an aqueous extract of licorice root, at 60–100 ml/day. SNMC is reported to have anti-inflammatory activity and has been used for the treatment of chronic viral hepatitis in Japan.[16]

In the retrospective study before 1996, two patients with deep hepatic coma on admission were treated with a combination therapy of CS and interferon (IFN). IFN β was administered at 3 million units/day. Cyclosporin A (CyA) was administered to one patient, and IFN monotherapy to one.

Serological markers

HBsAg, hepatitis B e antigen (HBeAg), anti-HBe antibody (HBeAb), HBcAb, IgM-HBc, IgM anti-HAV antibody, and anti-hepatitis D antibody were detected by commercial radioimmunoassay (Abbott Laboratories, Chicago, IL, USA), and HCV RNA was measured by nested reverse transcriptase-polymerase chain reaction.[17] Second generation anti-HCV antibody was measured by enzyme immunoassay (Ortho Diagnostics, Tokyo, Japan). IgM-EBV, IgM-CMV, and IgM-HSV were examined by enzyme-linked immunosorbent assay. Anti-nuclear antibody, anti-smooth muscle antibody, anti-mitochondrial antibody, and anti-liver kidney microsomal 1 antibody were examined by the fluorescent antibody method. HBV DNA polymerase was assayed according to the method of Kaplan et al.[18] The HBV DNA level was measured by hybridization assay (Abbott), branched DNA hybridization assay (Chiron, Emeryville, CA, USA), transcription-mediated amplification and hybridization protection assay (Chugai Diagnosis Science, Tokyo, Japan), or Amplicor HBV monitor (Roche Diagnostics, Tokyo, Japan).

Statistical analysis

Differences in proportions among the groups were compared by Fisher's exact probability test, Student's *t* test, or Welch's *t* test.

Results

Clinical features of severe chronic hepatitis B patients on admission

Of the 42 patients fulfilling the criteria of severe exacerbation, 29 were men and 13 were women. Mean age at the time of admission was 50.3 ± 13.5 years. Mean PT activity was 32 ± 14%, mean ALT was 820 ± 860 IU/l, and mean T-Bil was 12.1 ± 7.5 mg/dl. HBeAg/HBeAb status was +/– in 15, –/+ in 23, +/+ in two and –/– in two. Sixteen patients (38%) had primary diseases or conditions (five, non-Hodgkin's lymphoma; two, ulcerative colitis; and one each, acute lymphocytic leukemia, breast cancer, rheumatoid arthritis, pemphigoid, aplastic anemia, alcoholic hepatitis, HIV-positive, schizophrenia, Down's syndrome, and mental retardation), and 11 (26%) had been treated with immunosuppressive or cytotoxic drugs and suffered exacerbation after their withdrawal.

In the 1982–1996 period, the main treatment was delayed CS (period I), retrospectively. We defined the criteria of severe disease in 1997, and in the 1997–1998 period began to introduce CS as soon as possible after reaching a diagnosis; the main treatment was early CS (period II). After 1999, we administered LMV in combination with CS, and ETV after 2007; the main treatment was early CS and nucleoside analogs (NA) (period III). The three periods included 16, 10, and 16 patients, respectively (Table 1). Mean ages were 53.7 ± 14.0 years in 1982–1996, 43.0 ± 13.6 in 1997–1998, and 51.4 ± 12.0 in 1999–2007. Mean PT activities were 28 ± 14%, 33 ± 12%, and 36 ± 14%, mean ALT levels were 593 ± 853 IU/l, 1290 ± 1005, and 753 ± 693, and mean T-Bil levels were 15.8 ± 7.8 mg/dl, 9.9 ± 5.4, and 9.8 ± 7.2, respectively. Differences in age, sex, PT, ALT, HBe Ag/Ab status, and use of preimmunosuppressive or cytotoxic therapies for primary diseases were not significant in any of the periods. The T-Bil level was higher in period I than in period II ($P = 0.047$) or period III ($P = 0.03$). The time between the diagnosis of severe disease and the introduction of immunosuppressive drugs was longer in period I than in period II ($P = 0.02$) or period III ($P = 0.02$) (Table 1).

All surviving patients except one in period III were free of hepatic encephalopathy during the treatment course. Six patients (two in period I and four in period III) had hepatic encephalopathy at admission, and five did not respond to any therapy, including artificial liver supports such as plasma exchange and hemodiafiltration. One surviving patient in period III had grade II encephalopathy at admission. Twenty failed to respond to any therapy, including artificial liver supports, and gradually developed hepatic failure and died. One with mental retardation and recurrent pneumonia was treated with SNMC in period I and died of sepsis.

Therapies in each period

During 1982–1996, seven patients were treated with delayed CS, three with SNMC, two with delayed CS and IFN, two with early CS, one with CyA, and one with IFN. During 1997–1998, seven patients were treated with early CS, two with delayed CS, and one with delayed CS and LMV. During 1999–2007, seven patients were treated with early CS and LMV, two with early CS

Table 1. Clinical and biochemical features of patients according to study period

	1982–1996 (period I) Retrospective study	1997–1998 (period II) Prospective study	1999–2007 (period III) Prospective study
n	16	10	16
Age[a]	53.7 ± 14.0	43.0 ± 13.6	51.4 ± 12.0
Sex (M/F)	10/6	7/3	12/4
PT (%)[a]	28 ± 14	33 ± 12	36 ± 14
ALT (IU/l)[a]	593 ± 853	1290 ± 1005	753 ± 693
T-Bil (mg/dl)[a]	15.8 ± 7.8*	9.9 ± 5.4*	9.8 ± 7.2*
HBeAg/Ab	5/11[d]	5/5[d]	7/9[d]
Duration[a,b]	34.8 ± 43.0**	6.3 ± 5.4**	6.1 ± 4.1**
Pretherapy[c]	4	3	5
Recovery	4***	7***	10***

PT, prothrombin time; ALT, alanine aminotransferase; T-Bil, total bilirubin; HBeAg, hepatitis B e antigen; Ab, antibody
* $P = 0.047$, between periods I and II; $P = 0.03$ between periods I and III; Student's t test
** $P = 0.02$ between periods I and II, and between periods I and III; Welch's t test
*** $P = 0.03$ between periods I and II; $P = 0.04$ between periods I and III; Fisher's exact probability test
[a] Mean ± SD
[b] Time between the diagnosis of severe disease and introduction of corticosteroids
[c] Use of preimmunosuppressive or cytotoxic therapies for primary diseases
[d] Statistically not significant

Table 2. Therapies and clinical outcomes in each period

Period	Therapy Early CS	Non-early CS	Recovery rate
I. 1982–1996 (retrospective)			4/16
	Early CS		2/2
		Delayed CS	0/7
		Delayed CS + IFN	0/2
		Cyclosporine	0/1
		IFN	0/1
		SNMC	2/3
II. 1997–1998 (prospective)			7/10
	Early CS		7/7
		Delayed CS	0/2
		Delayed CS + LMV	0/1
III. 1999–2007 (prospective)			10/16
	Early CS + LMV		6/7
	Early CS + ETV		2/2
	Early CS + IFN, LMV		0/1
		Delayed CS + LMV	0/2
		LMV	1/2
		IFN, ADV	1/1
		SNMC	0/1

CS, corticosteroid; IFN, interferon; SNMC, stronger neominophagen C; LMV, lamivudine; ETV, entecavir; ADV, adefovir

and ETV, two with delayed CS and LMV, two with LMV, one with early CS, IFN, and LMV, one with IFN and ADV, and one with SNMC (Table 2).

Clinical outcome according to period

Overall, 21 (50%) of 42 patients survived. Four (25%) patients survived among those in 1982–1996 (period I), seven (70%) in 1997–1998 (period II), and ten (63%) in 1999–2007 (period III). The recovery rate was lower in period I than in period II ($P = 0.03$) or period III ($P = 0.04$) (Tables 1 and 2, Fig. 1).

Clinical outcomes according to therapy

In the retrospective study between 1982 and 1996, none of the ten patients survived who received delayed immunosuppressive therapy with or without IFN; two (100%) who received early CS, none who received IFN, and two (67%) who received SNMC survived. In the prospective

study between 1997 and 2007, 15 (88%) patients survived who were treated with early CS with or without antiviral drugs, but none of five who were treated with delayed CS with or without antiviral drugs, two (67%) who received antiviral drugs, and none who received SNMC (Table 2) survived.

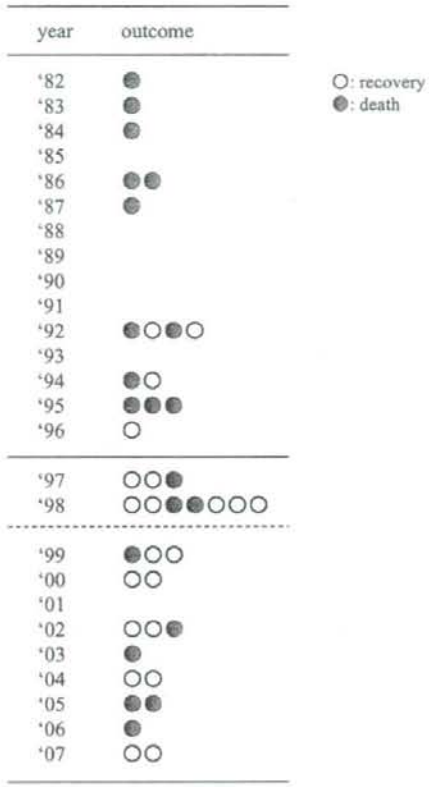

Fig. 1. Serial changes and clinical outcomes of patients

Comparison of CS monotherapy and CS and NA combination therapy in the prospective study

In the prospective study, the clinical features of patients treated with CS monotherapy and CS and NA combination therapy were compared. The differences in age, sex, PT, ALT, T-Bil, HBe Ag/Ab status, time between the diagnosis of severe disease and introduction of corticosteroids, and recovery rate were not significant between these groups (Table 3).

Clinical outcomes according to the time between the diagnosis of severe disease and the introduction of immunosuppressive drugs

Overall, immunosuppressive drugs were introduced to 19 patients within 10 days after the diagnosis of severe disease, and 17 (89%) recovered. In contrast, when they were introduced later than 10 days after the diagnosis of severe disease, none of 12 recovered (Fig. 2). The exact number of days before immunosuppressive drug introduction was not known in three patients, but they were obviously introduced later than 10 days after the diagnosis.

In the retrospective study between 1982 and 1996, both of two patients in whom immunosuppressive drugs were initiated within 10 days after the diagnosis of severe disease recovered, and all eight with initiation 11 days or longer after diagnosis died. In the prospective study between 1997 and 2007, 15 of 17 patients administered immunosuppressive drugs within 10 days after the diagnosis recovered, and all four treated 11 days or longer after diagnosis died.

Viral kinetics during therapies in CS and NA combination therapy

Among eight patients receiving early CS and NA therapy of the prospective study group, the hepatitis B

Table 3. Comparison of CS monotherapy and CS and NA combination therapy in the prospective study

	CS monotherapy	CS and NA combination therapy
n	9	13
Age[a]	44.6 ± 13.4	47.6 ± 13.3
Sex (M/F)	6/3	10/3
PT (%)[a]	34 ± 12	35 ± 14
ALT (IU/l)[a]	1392 ± 1010	863 ± 691
T-Bil (mg/dl)[a]	9.7 ± 5.7	8.2 ± 5.2
HBeAg/Ab	4/5	6/8
Duration[a,b]	6.3 ± 5.4	5.8 ± 4.0
Recovery	7	8

No statistically significant differences were observed
NA, nucleoside analog
[a] Mean ± SD
[b] Time between the diagnosis of severe disease and introduction of corticosteroids

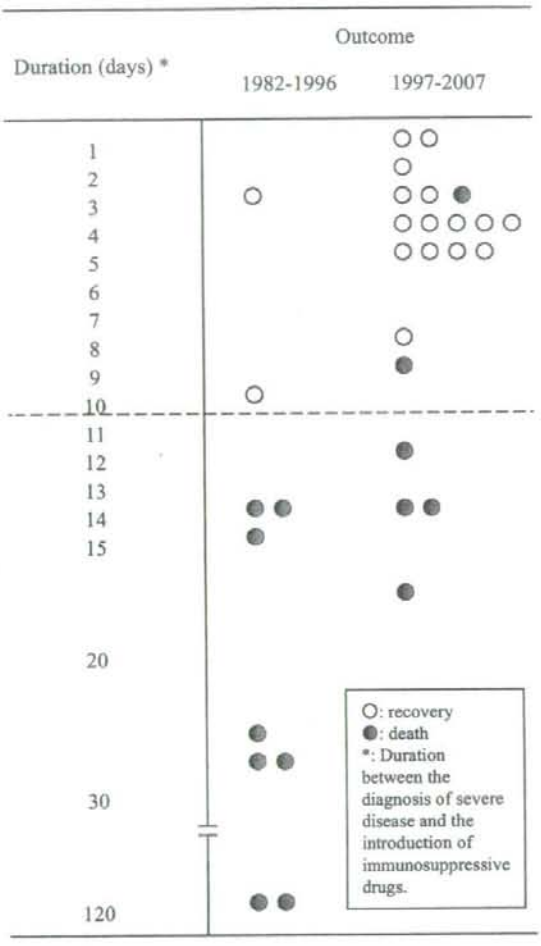

Fig. 2. Clinical outcomes according to the time between the diagnosis of severe disease and the introduction of immunosuppressive drugs

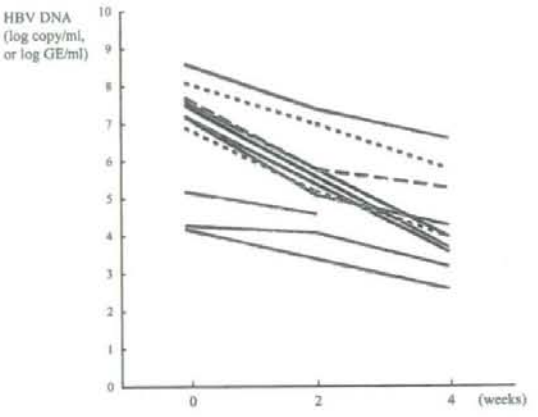

Fig. 3. Viral kinetics during therapies in the prospective study. *Solid*, *dashed*, and *dotted* lines denote early corticosteroid (CS) and nucleoside analog (NA) therapy, delayed CS and NA therapy, and NA therapy alone, respectively. *HBV*, hepatitis B virus; *GE*, genome equivalents

viral load was 6.5 ± 1.7 log copies/ml before treatment initiation (at week 0), 5.1 ± 1.2 at 2 weeks after treatment initiation, and 4.0 ± 1.3 at 4 weeks. The difference between the load at week 0 and at 4 weeks was significant ($P < 0.01$) (Fig. 3). Figure 3 shows the viral kinetics in the eight patients receiving early CS and NA therapy, one receiving delayed CS and NA therapy, and two receiving NA therapy.

Long-term outcomes of survivors

Of the nine survivors in the early CS group, two had LMV introduced afterward and three did not (four unknown), and corticosteroids were tapered to zero within a year in all five of these patients.

One patient with liver cirrhosis in the LMV group was introduced to ADV 2 years later, and the viral load decreased, but liver failure developed gradually. He received a living donor-liver transplantation from his son 3 years later.

Discussion

The prognosis of severe exacerbation of chronic hepatitis B is poor if signs of liver failure appear.[1,2,19] Recently, nucleoside analogs (NA), which exhibit a strong inhibitory effect on HBV replication, have been administered to patients with chronic hepatitis B, and dramatic improvements have been achieved. NA can be administered safely even to patients with severe disease,[10–15] but mortality is still high in patients demonstrating liver failure. A nationwide survey of fulminant hepatitis and late-onset hepatic failure between 1998 and 2003 in Japan revealed that the prognosis was especially poor in HBV carriers even after the introduction of LMV.[20] Tsubota et al.[21] reported that LMV monotherapy conferred no significant protection against rapid progression of the disease to liver failure in cases of severe acute exacerbation of chronic hepatitis B. Similar results were reported by Chan et al.[22] and Chien et al.[23] With the administration of NA, HBV DNA is reduced rapidly, but the improvement in liver function is delayed by a few weeks to a few months.[12] During the time lag, exces-

sive immunological reaction may continue and liver cell injury may progress. If in this phase effective therapeutic approaches were available, they would certainly be beneficial for these patients.

In our previous study, we described that the introduction of high-dose CS can reverse deterioration in patients with clinically severe, life-threatening exacerbation of chronic hepatitis B, when used in the early stage of illness.[9] We defined the criteria of severe disease in 1997, treated patients with severe disease with early initiation of CS of sufficient doses after 1997 prospectively, and after 1999 we used the combination of early and sufficient doses of CS and NA. In the present study, we examined the effect of the combination therapy of CS and NA.

One reason for not using CS for the treatment of chronic hepatitis B is that CS might enhance HBV replication through a steroid responsive element in the HBV genome.[24] In our previous study, none of the patients given high doses of CS showed increases in HBV replication during short-term observation periods.[9] In this study, HBV DNA decreased significantly during the 4-week period from the start of the CS and NA therapy. Gregory et al.[25] reported that in their study steroids would likely have proved beneficial if treatment had been started "much earlier" in the course of the illness. We are also convinced that timing is very important for optimum CS treatment.

In the 1982–1996 period, CS was introduced in the advanced stage of liver failure in most patients, but the recovery rate was low (25%). After we established the criteria of severe disease in 1997, CS was introduced at an earlier stage and the recovery rate improved (70% in 1997–1998). After we could use NA in combination with CS from 1999, we shortened the treatment period of CS while monitoring the viral load, but, contrary to our expectations, the recovery rate did not improve (63%).

Regarding the timing of the therapies, none of the patients recovered when delayed immunosuppressive therapy, with or without antiviral drugs such as NA, were implemented. In contrast, 89% of patients who received early immunosuppressive therapy with or without NA recovered ($P < 0.001$). The importance of the early introduction of immunosuppressive therapy was shown again in the presence of effective NA. Regarding the time between the diagnosis of severe disease and the start of immunosuppressive therapy, 89% of patients administered immunosuppressive therapy within 10 days recovered, but none administered the therapy 11 days or longer after diagnosis recovered. When the start of the treatment is delayed beyond 10 days, large numbers of hepatocytes are likely already destroyed and inhibition of the inflammatory reaction might not be effective. Our cutoff point of 10 days to define "early introduction" seems to be very close to the mark.

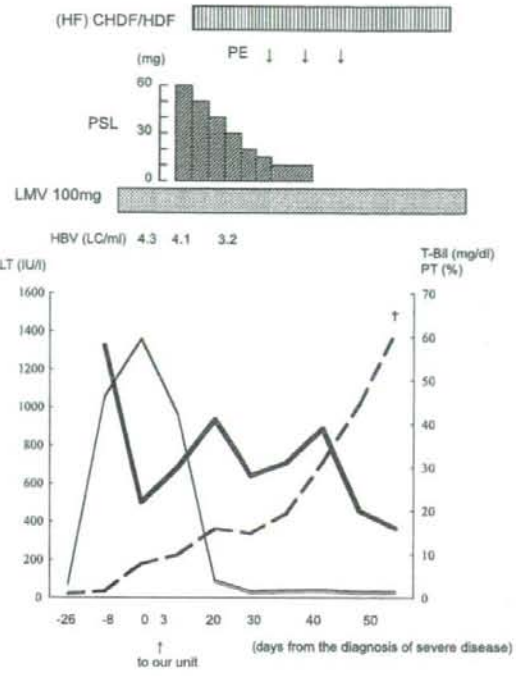

Fig. 4. Clinical course of a 63-year-old female patient. She suffered from chronic hepatitis B with hepatitis B e antigen (HBeAg). She had natural exacerbation, and lamivudine (*LMV*) was administered before the criteria of severe disease were fulfilled. Eight days after the start of LMV, she showed hepatic encephalopathy, marked prolonged prothrombin time (*PT*) activity, and elevated alanine transaminase (*ALT*) and total bilirubin (*T-Bil*). A corticosteroid was administered 9 days after the diagnosis of severe disease, but she did not respond to the therapies. *Thick solid*, *thin solid*, and *dashed lines* denote PT, ALT, and T-Bil, respectively. *PE*, plasma exchange; *(HF) CHDF*, (high flow) continuous hemodiafiltration; *HDF*, hemodiafiltration; *PSL*, prednisolone

Two patients died despite early CS and NA. One was an HIV-positive man who was hospitalized 10 days after the onset of jaundice. Three days after the diagnosis of severe disease, CS and NA were administered, but he did not respond to the therapy. The other was a woman suffering from acute exacerbation in a related hospital, and LMV was administered before the criteria of severe disease were fulfilled. Eight days after the start of LMV, she showed hepatic encephalopathy, marked prolonged PT activity, and elevated ALT and T-Bil. CS was administered 9 days after the diagnosis of severe disease, but she did not respond to the therapy. In both cases, the timing of the diagnosis of severe disease was delayed,

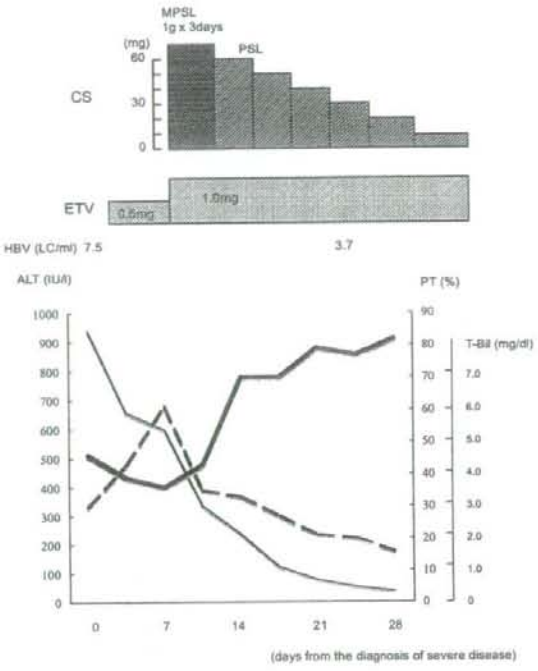

Fig. 5. Clinical course of a 51-year-old male patient. He suffered from chronic hepatitis B with HBeAg. He had natural exacerbation, and entecavir (*ETV*) was administered the day after admission, but he did not show a trend toward remission in PT or T-Bil. A double dose of ETV, together with CS, were introduced 5 days after the diagnosis of severe disease, and he responded to the therapy. *Thick solid*, *thin solid*, and *dashed lines* denote PT, ALT, and T-Bil, respectively. *MPSL*, methylprednisolone

although CS and NA therapy was started within 10 days after this diagnosis. These results emphasize that an even earlier diagnosis of severe disease is required.

Our results highlight the importance of immunosuppressive therapy for preventing the progression to liver failure. As Tsubota et al.[21] reported that an effective therapeutic strategy should be aggressively combined with LMV because LMV lacks the capability of suppressing a hyperimmune reaction, it seems that antiviral therapy is not sufficient to stop progressive deterioration and additional therapy to suppress liver cell degeneration may be necessary. Combination treatment with early high-dose CS and NA might be effective in suppressing the excessive host immune response in the early period. Additionally, NA can make it possible to shorten the term of CS therapy.

In summary, our study demonstrated that the early introduction of high-dose CS treatment in combination with NA may be beneficial for cases of clinically severe acute exacerbation of chronic hepatitis B. We were unable to include placebo-controlled patients, considering the current knowledge of the poor prognosis of such patients and our historical control patients between 1982 and 1996. Nevertheless, delay in treatment may result in fatal liver failure even when these treatment protocols are used, suggesting that an early diagnosis of such patients is urgently required.

Acknowledgments. We are indebted to all our colleagues at the liver units of our hospitals who cared for the patients described herein. In addition, we thank Dr. Toshiki Ehata (Ehata Clinic), Dr. Kazuo Suzuki (Takushinkai Tatsumi Hospital) and Professor Masao Omata (University of Tokyo) for clinical contributions.

References

1. Editorial. Chemotherapy and hepatitis B. Lancet 1989;ii:1136–7.
2. Nakamura Y, Motokura T, Fujita R, Yamashita T, Ogata E. Severe hepatitis related to chemotherapy in hepatitis B virus carriers with hematologic malignancies. Cancer 1996;78:2210–5.
3. Chisari FV, Ferrari C. Hepatitis B virus immunopathogenesis. Annu Rev Immunol 1995;13:29–60.
4. Sjogren MH, Hoofnagle JH, Waggoner JG. Effect of corticosteroid therapy on levels of antibody to hepatitis B core antigen in patients with chronic type B hepatitis. Hepatology 1987;7:582–5.
5. Lam KC, Lai CL, Trepo C, Wu PC. Deleterious effect of prednisolone in HBsAg-positive chronic active hepatitis. N Engl J Med 1981;304:380–6.
6. Hoofnagle JH, Davis GL, Pappas SC, Hanson RG, Peters M, Avigan MI, et al. A short course of prednisolone in chronic type B hepatitis. Ann Intern Med 1986;104:12–7.
7. Scullard GH, Smith CI, Merigan TC, Robinson WS, Gregory PB. Effects of immunosuppressive therapy on viral markers in chronic active hepatitis B. Gastroenterology 1981;81:987–91.
8. Lau JY, Bird GL, Gimson AE, Alexander GJ, Williams R. Treatment of HBV reactivation after withdrawal of immunosuppression. Lancet 1991;337:802.
9. Fujiwara K, Yokosuka O, Kojima H, Kanda T, Saisho H, Hirasawa H, et al. Importance of adequate immunosuppressive therapy for the recovery of patients with "life-threatening" severe exacerbation of chronic hepatitis B. World J Gastroenterol 2005;11:1109–14.
10. Santantonio T, Mazzola M, Pastore G. Lamivudine is safe and effective in fulminant hepatitis B. J Hepatol 1999;30:551–3.
11. Van Thiel DH, Friedlander L, Kania RJ, Molloy PJ, Hassanein T, Wahlstrom E, et al. Lamivudine treatment of advanced and decompensated liver disease due to hepatitis B. Hepatogastroenterology 1997;44:808–12.
12. Chan TM, Wu PC, Li FK, Lai CL, Cheng IK, Lai KN. Treatment of fibrosing cholestatic hepatitis with lamivudine. Gastroenterology 1998;115:177–81.
13. ter Borg F, Smorenburg S, de Man RA, Rietbroek RC, Chamuleau RA, Jones EA. Recovery from life-threatening corticosteroid-unresponsive chemotherapy-related reactivation of hepatitis B associated with lamivudine therapy. Dig Dis Sci 1998;43:2267–70.
14. Ahmed A, Keeffe EB. Lamivudine therapy for chemotherapy-induced reactivation of hepatitis B virus infection. Am J Gastroenterol 1999;94:249–51.
15. Villeneuve JP, Condreay LD, Willems B, Pomier-Layrargues G, Fenyves D, Bilodeau M, et al. Lamivudine treatment for decompensated cirrhosis resulting from chronic hepatitis B. Hepatology 2000;31:207–10.

16. Iino S, Tango T, Matsushima T, Toda G, Miyake K, Hino K, et al. Therapeutic effects of stronger neo-minophagen C at different doses on chronic hepatitis and liver cirrhosis. Hepatol Res 2001; 19:31–40.
17. Okamoto H, Okada S, Sugiyama Y, Tanaka T, Sugai Y, Akahane Y, et al. Detection of hepatitis C virus RNA by a two-step polymerase chain reaction with two pairs of primers deduced from the 5'-noncoding region. Jpn J Exp Med 1990;60:215–22.
18. Kaplan PM, Greenman RL, Gerin TL, Purcell RH, Robinson WS. DNA polymerase associated with human hepatitis B antigen. J Virol 1973;12:995–1005.
19. Markovic S, Drozina G, Vovk M, Fidler-Jenko M. Reactivation of hepatitis B but not hepatitis C in patients with malignant lymphoma and immunosuppressive therapy. A prospective study in 305 patients. Hepatogastroenterology 1999;46:2925–30.
20. Fujiwara K, Mochida S, Matsui A, Nakayama N, Nagoshi S, Toda G; Intractable Liver diseases Study Group of Japan. Fulminant hepatitis and late onset hepatic failure in Japan. Hepatol Res 2008;38:646–57.
21. Tsubota A, Arase Y, Suzuki Y, Suzuki F, Sezaki H, Hosaka T, et al. Lamivudine monotherapy for spontaneous severe acute exacerbation of chronic hepatitis B. J Gastroenterol Hepatol 2005;20:426–32.
22. Chan HL, Tsang SW, Hui Y, Leung NW, Chan FK, Sung JJ. The role of lamivudine and predictors of mortality in severe flare-up of chronic hepatitis B with jaundice. J Viral Hepat 2002;9:424–8.
23. Chien RN, Lin CH, Liaw YF. The effect of lamivudine therapy in hepatic decompensation during acute exacerbation of chronic hepatitis B. J Hepatol 2003;38:322–7.
24. Tur-Kaspa R, Burk RD, Shaul Y, Shafritz DA. Hepatitis B virus DNA contains a glucocorticoid-responsive element. Proc Natl Acad Sci USA 1986;83:1627–31.
25. Gregory PB, Knauer M, Kempson RL, Miller R. Steroid therapy in severe viral hepatitis. N Engl J Med 1976;294:681–7.

Arch Virol (2008) 153:1575–1579
DOI 10.1007/s00705-008-0143-5

BRIEF REPORT

A mutational shift from domain III to II in the internal ribosome entry site of hepatitis C virus after interferon–ribavirin therapy

Kei Ogata · Takahito Kashiwagi · Jun Iwahashi · Koyu Hara · Haruhito Honda · Tatsuya Ide · Ryukichi Kumashiro · Michinori Kohara · Michio Sata · Hiroshi Watanabe · Nobuyuki Hamada

Received: 7 August 2007 / Accepted: 21 May 2008 / Published online: 1 July 2008

Abstract We focused on the relationship between variation in the IRES of hepatitis C virus (HCV) genotype 1b and clinical outcome, since the internal ribosome entry site (IRES) has a comparatively low heterogeneity and it might be easy to find unique substitutions. Patients infected with HCV were selected using strict criteria, and unique mutations in the IRES were extracted by the subtraction of common mutations. We found that most mutations accumulated in domain III (dIII) of IRES in sustained virological responders (SVRs) and non-SVRs before therapy. However, these mutations were exclusively observed in domain II (dII) in non-SVR at 2 weeks after the start of therapy.

Hepatitis C virus (HCV) is an enveloped RNA virus of the genus *Hepacivirus* in the family *Flaviviridae* [2]. The genomic RNA is a plus strand consisting of approximately 9,600 nucleotides, which contains a large open reading frame and two untranslated regions. The untranslated regions (UTR) are present at each end of the genome (5′ and 3′ termini) and are involved in not only the translation of viral proteins but also genomic replication. An especially highly conserved region (about 341 nucleotides) in the 5′ UTR is known to act as an internal ribosome entry site (IRES), which is essential for the translation of viral proteins [17, 18]. The IRES forms a tertiary structure for ribosome binding and subsequent protein synthesis [4]. An artificial alteration of the sequence can severely affect translational activity [6].

HCV is a significant cause of morbidity and mortality, infecting over 170 million people worldwide. The majority (about 80%) of individuals with HCV infection develop chronic hepatitis, which can progressively lead to cirrhosis (10–20%), and eventually to hepatocellular carcinoma (5%). Despite recent efforts, the current therapy [pegylated-interferon (IFN) with ribavirin] for HCV infection remains inadequate for approximately half of all patients. The mechanisms of tolerance against this therapy are still unknown. HCV is genetically heterogeneous, and it circulates as a population of closely related genomes, referred to as quasispecies [12]. Previous studies have shown that specific regions in the HCV genome, such as the IRES and the NS5B coding region, accumulate nucleotide substitutions in patients receiving antiviral therapy [8, 16]. These results suggest that some mutants show tolerance against the current therapy. However, the significance of genetic variations in these regions is still not fully understood either biochemically or clinically. The quasispecies, which normally appear during treatment, consist of many heterogeneous clones. This heterogeneity makes the interpretation of the relationship between clinical outcome and resistance mutations difficult.

K. Ogata · T. Kashiwagi · J. Iwahashi · K. Hara · H. Honda · H. Watanabe · N. Hamada (✉)
Division of Infectious Diseases, Department of Infectious Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan
e-mail: nhamada@med.kurume-u.ac.jp

K. Ogata · T. Ide · R. Kumashiro · M. Sata
Division of Gastroenterology, Department of Internal Medicine, Kurume University School of Medicine, 67 Asahi-machi, Kurume, Fukuoka 830-0011, Japan

M. Kohara
Department of Microbiology and Cell Biology, The Tokyo Metropolitan Institute of Medical Biology, 3-18-22 Honkomagome, Bunkyo-ku, Tokyo 113-8613, Japan

In this study, we examined the relationship between the IRES of HCV from the pre- and in-treatment sera of the patients (sustained virological responder (SVR) and non-SVR to IFN–ribavirin therapy) and its clinical outcome, because there is said to be a comparatively lower mutant spectrum complexity in the IRES in comparison to the coding region. Previous reports have suggested that no clinically significant variations exist in the IRES [16, 19]. However, we found a significant importance of IRES mutations in resistant HCV clones by selecting patients carefully and isolating the specific mutations for SVRs or non-SVRs.

Among the patients hospitalized at the Kurume University Hospital from 2001 to 2003, seven patients demonstrating HCV genotype 1b with a high viral load (>100 Kilo International Units/ml (KIU/ml) by Amplicor-HCV monitor ver. 2; Roche Molecular Diag. Co., Tokyo, Japan) were included in this study. These patients were carefully selected according to the selection standard, i.e. the patients underwent the standard treatment protocol of IFN–ribavirin therapy. There were no patients with a reduction of drug or a discontinuation of the therapy. Informed consent for this therapy was obtained from every patient, and the study was conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki. We measured the amount of HCV RNA from patients' sera regularly during the treatment for 6 months (Fig. 1) and divided them into two groups, SVR and non-SVR. An SVR was defined as a patient in whose serum HCV RNA was not detected for at least 6 months after the end of the treatment. A non-SVR was defined as a patient in whom HCV RNA levels were reduced slightly but a high level was retained in the serum within 6 months of the end of the treatment. Three of the patients (Pt4, Pt5, and Pt6) were SVRs, while the other patients (Pt1, Pt2, Pt3, and Pt7) were non-SVRs. The HCV RNA levels in the SVRs decreased dramatically to around the lower detection limit within a week. Although the HCV RNA levels in non-SVRs decreased slowly, they remained high for at least 2 weeks.

In order to address the question of whether the IRES correlates with the clinical outcome of HCV, we compared the nucleotide sequence of the IRES between the SVRs and non-SVRs. The patients' sera were collected at pre-treatment 0 and 2 weeks after the start of therapy. Viral RNA was extracted from the serum, and IRES cDNA was obtained by nested RT-PCR. To detect all variations of the IRES, primer cocktail (5′-GCACACCCAACCTGGGGC CC-3′, 5′-CGAGGTTGCGACCGCTCGGAAG-3′, 5′-GA AGCCGCATGTGAGGGTATCGATGAC-3′) was used for reverse transcription. PCR was performed for 35 cycles (94°C, 30 s; 55°C, 30 s; 72°C, 60 s) after pre-heating (94°C, 2 min) using an outer primer pair (5′-GGGGCGA CACTCCACCATAG-3′, 5′-GATCTGACCACCGCCCGG

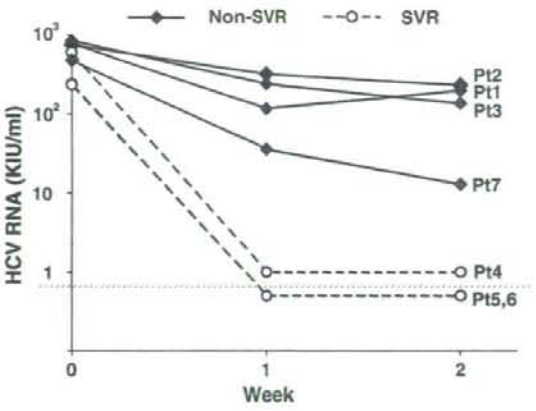

Fig. 1 Viral RNA kinetics in chronically HCV-infected patients undergoing interferon–ribavirin therapy. The *solid* and *dashed lines* indicate the kinetics of the amount of RNA from non-SVRs (*solid diamond*) and SVRs (*open circle*), respectively. The *dashed lines* from two patients' data at the bottom are overlapping. *Horizontal dotted line* shows the limit of detection by Amplicor-HCV monitor ver. 2. All patients received intramuscular IFNα-2b (Intron, Schering-Plough, Kenilworth, NJ) in combination with a daily oral 600–800 mg dose of ribavirin for 24 weeks. For the first 2 weeks of the combination therapy, 6 MU of IFNα-2b were given daily. The IFN dosing frequency was then reduced to 6 MU three times a week for the remaining 22 weeks. The ribavirin dosage was 600 mg daily for the patients who weighed less than 60 kg and 800 mg daily for patients who weighed between 60 and 80 kg

GAAC-3′), and then incubated at 72°C for 10 min. This cycle was again performed under the same conditions using an inner primer pair (5′-GTTTTTCTTTGAGGTTTAGG-3′, 5′-ACACTCCACCATAGATCACTC-3′). The final PCR product of 352 bp was cloned using TA cloning vector (pT7Blue-2, Novagen, USA), and three independent clones from each patient were sequenced (sequencer Model 310, ABI, USA).

IRES sequences of SVRs at 1 and 2 weeks after the start of therapy were unavailable because the amount of RNA was below or near the detection limit, and RNA could not be isolated, although the RNAs were detected by Amplicor-HCV monitor ver. 2, which is one of the qualitative assays with high sensitivity. The mutations specific to the non-SVRs, at pre-treatment (0W) and 2 weeks after the start of therapy (2W), are shown in Fig. 2. The distribution of mutations is summarized in Table 1, including the mutations specific to the SVRs at pre-treatment (0W). For pre-treatment (0W), the distribution of mutations was similar when SVRs and non-SVRs were compared (Table 1), with the exception that mutations were also found in dII in all clones from patient 3 (non-SVR). Previous therapy with IFN might be related to mutation in dII, since patient 3 has a history of IFN monotherapy. In comparison with pre-treatment (0W) and 2 weeks (2W)

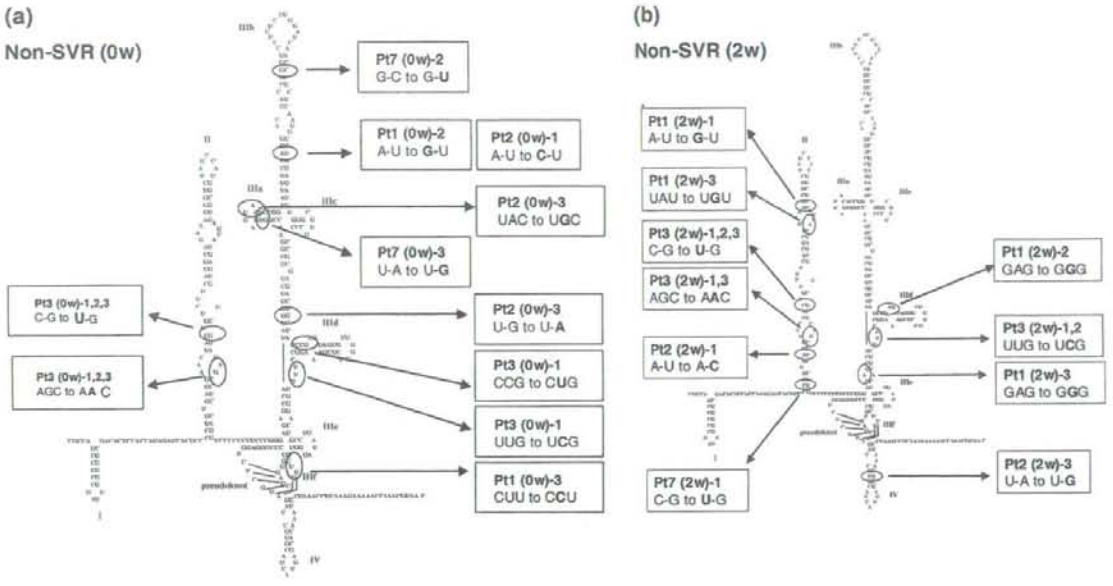

Fig. 2 The nucleotide sequence and predicted secondary structure of the HCV IRES. The nucleotide sequence shows the consensus sequence which was found among SVRs 0 weeks, non-SVRs 0 weeks and non-SVRs 2 weeks. The secondary structure is based on the HCV-JS strain [5]. The original structure is not changed for simplification of the figure, although several base pairings are partly disrupted due to the mutations. The numbers following Pt indicate the patients and each clone number from same patient. The number in brackets shows the week after the start of treatment. The position of mutations found is indicated in the *circle*. **a** Non-SVR-specific heterogeneity at pre-treatment 0 weeks. **b** Non-SVR-specific heterogeneity at 2 weeks after the start of therapy

Table 1 Number of mutations in non-SVRs and SVRs

		SVRs	Non-SVRs	
		0 weeks[a]	0 weeks	2 weeks
dII	UM[b]	0	1	4
	NM	0	1	2
	SM	0	0	0
	Total	0	2	6
dIII	UM	4	5	0
	NM	3	3	3
	SM	0	1	0
	Total	7	9	3
dIV	UM	0	0	1
	NM	1	0	0
	SM	0	0	0
	Total	1	0	1

[a] Week after the start of therapy

[b] *UM* unstable mutation, *NM* null mutation or *SM* (stable mutation) mean mutations that would disrupt the base pairing, not affect the base pairing, or form potential base pairing, respectively

after the start of therapy, in non-SVRs, the number of mutations in dII increased from 2 to 6 (Table 1, and compare Fig. 2a, b). Conversely, the number of mutations in dIII decreased from 9 to 3. These results indicate that mutation was preferentially shifted from dIII to II in non-SVRs during the therapy. It was also noted that the number of mutations which would disrupt the base pairing (referred to as UM in Table 1) in dII increased from 1 to 4, whereas those in dIII decreased from 5 to 0 (Table 1). This suggests that the mutational shift from dIII to II might disrupt several base pairings in dII but restore the base pairing in dIII. Non-SVR clones at 2 weeks after the therapy showed a decreased translational activity using a luciferase reporter gene assay (data not shown). This indicates that the mutational shift from dIII to II in non-SVRs leads to a decrease in viral translational activity.

Recently, comparing the IRES sequences of non-SVRs with those of SVRs, a few specific nucleotide substitutions have been observed [14, 16, 19]. No clinically significant variations have been reported in the IRES. However, we found a relationship between IRES mutations and clinical outcome. The discrepancy between previous reports and our results may be ascribed to several methodological differences. First, we selected the patients in order to obtain a uniform background of patients using strict criteria. Second, we extracted unique mutations specific for SVRs or non-SVRs in order to identify the principal mutations. Our study demonstrates that mutation was preferentially

shifted from dIII to II in non-SVRs during IFN–ribavirin therapy. Moreover, a similar tendency of mutational shift was observed with patients currently undergoing therapy with PegIFN–ribavirin (data not shown), suggesting a relationship between IRES mutation and clinical outcome.

It is apparent that the accumulation of unstable mutations in dIII shifts to that of unstable mutations in dII during treatment (Table 1). The same result was also obtained using a new IRES structure model [10] instead of using the previous model described in this study (Fig. 2). dIII has been reported to directly bind to the ribosome to stabilize the IRES-ribosome complex, whereas dII is involved in triplet decoding and therefore does not bind to the ribosome directly [9, 15]. It is thus possible that a mutation in dIII rather than dII may dramatically change the ribosome binding activity, e.g. the release of the IRES from the ribosome, and the degradation of free IRES and HCV RNA [11] or vice versa. Why does the dIII mutant in SVRs and non-SVRs accumulate before treatment? It may be partly because dIII mutant has a dominant trait, e.g. the translational activity of the dIII mutant is higher than that of the dII mutant (data not shown). And why are dIII mutants in SVRs and non-SVRs sensitive to therapy? dIII mutant may exist at the error threshold point, as suggested by the error catastrophe theory regarding viruses [1]. Therefore, if drugs that perturb the viral load, e.g. ribavirin and IFN, are added, then the clone may rapidly disappear with, for example, catastrophic breakdown of the ribosome-dIII complex. On the contrary, dII mutant may have recessive trait, e.g. inefficient translational activity (data not shown). However, this trait, conversely, might be an advantage for escape from the immune system, because inefficient translational activity might lead to a reduction in the number of viral proteins that are recognized by immune cells. An HCV variant containing a dIII deletion in the IRES, described in a previous report [13], might be the extreme escape mutant.

It has generally been thought that the decay curve of HCV in patients undergoing combination therapy with IFN–ribavirin basically exhibits a biphasic pattern in both SVRs and non-SVRs [3]. The decay curve of HCV in this study also seems to be biphasic: the first phase with a rapid decrease during 0–7 days, and the second phase with a slower decrease after 7 days (Fig. 1) except Pt1 (non-SVR). HCV in patients is a mixture of genetically distinct variants known as quasispecies. We speculate that HCV is composed of several variants before therapy, including mutations in dII that are resistant to therapy and in dIII that are sensitive to therapy. Particularly in non-SVRs, dIII mutant might be predominant and excluded rapidly in the first phase, whereas the dII mutants may exist as a small population in the first phase, resist the therapy, become a predominant population, and show slower decrease in the second phase. Theoretically, it is unlikely that a dII mutant would be directly derived from a dIII mutant, because such mutants would require simultaneous mutations in a short period: firstly a preferential mutation in dII, and secondly a selective mutation which reverts to the original dIII. On the other hand, in SVRs, the population size of the dIII mutant would be larger than that of the non-SVRs before therapy, and thus the decay curve shows a more rapid decrease than that of the non-SVRs. The dII mutant in SVRs may be a much smaller population than that in non-SVRs, possibly below or near the detection limit by PCR.

We propose that dII might be a potential target for antiviral therapy to improve long-lasting therapy. Indeed, siRNAs specific for highly conserved regions of HCV, including dII, inhibited virus translation and subgenomic replication in cultured cells [7]. The siRNA specific for the mutated position in dII might suppress dII mutant clones and shorten the length of therapy. Also, a determination of the IRES sequence in the dII mutant before therapy should be useful for the prediction of drug response and rapid design of siRNAs.

Acknowledgments We would like to thank to T. Sayano (Kyushu University), K. Yasuda (Department of Parasitology, Kurume University School of Medicine) and Y. Imamura (Division of Infectious Diseases, Department of Infectious Medicine, Kurume University School of Medicine) for their valuable technical assistance. We also thank T. Omura for his critical reading of the manuscript.

References

1. Eigen M (2002) Error catastrophe and antiviral strategy. Proc Natl Acad Sci USA 99:13374–13376
2. Fauquet CM, Mayo MA, Maniloff J, Desselberger U, Ball LA (2005) Virus taxonomy. Eighth Report of the International Committee on Taxonomy of Viruses, Elsevier Academic Press, London
3. Herrmann E, Zeuzem S (2006) The kinetics of hepatitis C virus. Eur J Gastroenterol Hepatol 18:339–342
4. Honda M, Ping LH, Rijnbrand RC, Amphlett E, Clarke B, Rowlands D, Lemon SM (1996) Structural requirements for initiation of translation by internal ribosome entry within genome-length hepatitis C virus RNA. Virology 222:31–42
5. Honda M, Beard MR, Ping LH, Lemon SM (1999) A phylogenetically conserved stem-loop structure at the 5′ border of the internal ribosome entry site of hepatitis C virus is required for cap-independent viral translation. J Virol 73:1165–1174
6. Kieft JS, Zhou K, Jubin R, Murray MG, Lau JY, Doudna JA (1999) The hepatitis C virus internal ribosome entry site adopts an ion-dependent tertiary fold. J Mol Biol 292:513–529
7. Korf M, Jarczak D, Beger C, Manns MP, Kruger M (2005) Inhibition of hepatitis C virus translation and subgenomic replication by siRNAs directed against highly conserved HCV sequence and cellular HCV cofactors. J Hepatol 43:225–234
8. Kumagai N, Takahashi N, Kinoshita M, Tsunematsu S, Tsuchimoto K, Saito H, Ishii H (2004) Polymorphisms of NS5B protein relates to early clearance of hepatitis C virus by interferon plus ribavirin: a pilot study. J Viral Hepat 11:225–235

9. Lukavsky PJ, Otto GA, Lancaster AM, Sarnow P, Puglisi JD (2000) Structures of two RNA domains essential for hepatitis C virus internal ribosome entry site function. Nat Struct Biol 7:1105–1110
10. Lukavsky PJ, Kim I, Otto GA, Puglisi JD (2003) Structure of HCV IRES domain II determined by NMR. Nat Struct Biol 10:1033–1038
11. Lytle JR, Wu L, Robertson HD (2001) The ribosome binding site of hepatitis C virus mRNA. J Virol 75:7629–7636
12. Martell M, Esteban JI, Quer J, Genesca J, Weiner A, Esteban R, Guardia J, Gomez J (1992) Hepatitis C virus (HCV) circulates as a population of different but closely related genomes: quasispecies nature of HCV genome distribution. J Virol 66:3225–3229
13. Revie D, Alberti MO, Braich RS, Bayles D, Prichard JG, Salahuddin SZ (2006) Discovery of significant variants containing large deletions in the 5′UTR of human hepatitis C virus (HCV). Virol J 3:82
14. Soler M, Pellerin M, Malnou CE, Dhumeaux D, Kean KM, Pawlotsky JM (2002) Quasispecies heterogeneity and constraints on the evolution of the 5′ noncoding region of hepatitis C virus (HCV): relationship with HCV resistance to interferon-alpha therapy. Virology 298:160–173
15. Spahn CM, Kieft JS, Grassucci RA, Penczek PA, Zhou K, Doudna JA, Frank J (2001) Hepatitis C virus IRES RNA-induced changes in the conformation of the 40s ribosomal subunit. Science 291:1959–1962
16. Thelu MA, Drouet E, Hilleret MN, Zarski JP (2004) Lack of clinical significance of variability in the internal ribosome entry site of hepatitis C virus. J Med Virol 72:396–405
17. Tsukiyama-Kohara K, Iizuka N, Kohara M, Nomoto A (1992) Internal ribosome entry site within hepatitis C virus RNA. J Virol 66:1476–1483
18. Wang C, Sarnow P, Siddiqui A (1993) Translation of human hepatitis C virus RNA in cultured cells is mediated by an internal ribosome-binding mechanism. J Virol 67:3338–3344
19. Yamamoto C, Enomoto N, Kurosaki M, Yu SH, Tazawa J, Izumi N, Marumo F, Sato C (1997) Nucleotide sequence variations in the internal ribosome entry site of hepatitis C virus-1b: no association with efficacy of interferon therapy or serum HCV-RNA levels. Hepatology 26:1616–1620

Dig Dis Sci (2008) 53:2999–3006
DOI 10.1007/s10620-008-0384-x

ORIGINAL ARTICLE

Predictive Factors Associated with the Progression to Hepatic Failure Caused by Lamivudine-Resistant HBV

Reiichiro Kuwahara · Ryukichi Kumashiro · Tatsuya Ide · Yuriko Koga · Teruko Hino · Akiko Hisamochi · Kazuo Tanaka · Kei Ogata · Hiroyuki Koga · Yukari Takao · Michio Sata

Received: 17 May 2005 / Accepted: 12 July 2005 / Published online: 10 July 2008

Abstract The aims of this study were to select the patients with a potential for progression to hepatic failure due to lamivudine-resistant HBV and to standardize the treatment for patients with lamivudine-resistant HBV. Patients ($n = 47$) with reactivated hepatitis due to lamivudine-resistant HBV were classified into two groups, with and without potential for progression to hepatic failure, according to the criteria using the data of serum bilirubin level and prothrombin activity after the reactivated hepatitis. Multivariate analysis showed that prothrombin activity at the initiation of lamivudine therapy was related to the deterioration of the liver function after the emergence of lamivudine-resistant HBV ($P = 0.0025$, 95%CI 0.8269–0.9601). We assume that earlier additional or substitutive treatment with other antiviral agent, such as adefovir dipivoxil, should be recommended when the lamivudine-resistant HBV is detected in patients with the history of decompensated liver disease before the administration of lamivudine, even when hepatitis has not been reactivated yet.

Keywords Hepatitis B virus · Liver cirrhosis · Lamivudine · Adefovir dipivoxil · Hepatic failure · YMDD motif

R. Kuwahara (✉) · R. Kumashiro · T. Ide · Y. Koga · T. Hino · A. Hisamochi · K. Tanaka · K. Ogata · H. Koga · Y. Takao · M. Sata
Kurume University School of Medicine,
Kurume 830-0011, Japan
e-mail: ray@med.kurume-u.ac.jp

Introduction

Hepatitis B virus (HBV) infection remains a major global health problem. Chronic HBV infection is one of the major causes of cirrhosis and hepatocellular carcinoma in endemic areas, causing more than 1 million deaths per year [1].

Until recently, interferon (IFN) was the only effective antiviral agent for patients with chronic HBV infection. Although treatment with IFN benefits some patients [2–4], the overall response rate is less than 40% [3, 5, 6]. IFN treatment is occasionally contraindicated in patients with liver cirrhosis because of the risk of potentially life-threatening complications [7, 8]. Liver transplantation with antiviral prophylaxis is available as a potential salvage therapy for some patients [9], but this is possible only in a limited number of countries and not available in the rest of the world where HBV is endemic [1].

Recently, lamivudine has become the main therapeutic option for the treatment of chronic HBV infection. Lamivudine is a potent inhibitor of HBV replication by suppressing HBV-DNA polymerase. Lamivudine leads to a rapid and profound decrease in serum HBV-DNA levels, reduces disease activity, and increases the rate of hepatitis B e (HBe)-seroconversion significantly with few adverse events [10, 11]. It is also reported that treatment with lamivudine induces histological improvement [12]. Although lamivudine is a great benefit for patients with chronic HBV infection, the emergence of lamivudine-resistant HBV is the major drawback of lamivudine treatment. Lamivudine-resistant HBV strains contain methionine (M) to isoleucine (I) or valine (V) substitutions in the YMDD motif in the C-domain of the RNA-dependent DNA polymerase [13, 14]. In recent clinical trials, the emergence of lamivudine-resistant HBV occurs in 14–32% of patients after 1 year of therapy [9, 10]. Although longer treatment with lamivudine

increases the rate of HBe-seroconversion, prolonged treatment with lamivudine has been associated with the emergence of lamivudine-resistant HBV with mutations in the YMDD motif [15]. The incidence of YMDD variants was up to 74% after 4 years of lamivudine therapy [16]. The clinical course after the selection of lamivudine-resistant HBV seems to be benign [17]. The variant HBV is replication-incompetent compared with the wild-type HBV [18]. HBV-DNA levels and alanine aminotransferase (ALT) levels remained lower by comparison with baseline values in the majority of patients with lamivudine-resistant HBV. However, in some literature, it has been reported that hepatic failure and mortality developed after the emergence of lamivudine-resistant HBV [19–22]. Therefore, it should be significant clinically to select patients with the risk of progression to hepatic failure, and it may be possible to prevent reactivated hepatitis from progressing to a fatal disease if the proper countermeasures are taken before the development of hepatic failure. In order to prevent reactivated hepatitis progressing to a fatal disease, we should select patients with the potential for developing hepatic failure due to lamivudine-resistant HBV among all patients who are treated with lamivudine.

However, there is little information about the predictive factors associated with the development of hepatic failure after the emergence of lamivudine-resistant HBV. For the purpose of detecting the predictive factors associated with the progression of the disease, we retrospectively analyzed the clinical and virological characteristics of patients with the viral breakthrough.

Methods

Patients

Forty-seven patients who were treated with lamivudine because of HBV-related chronic liver disease were studied. None of the patients enrolled in this study were treated with an antiviral agent such as IFN or other nucleoside analogues within 3 years of the initiation of the treatment with lamivudine. The patients were followed up every 2 weeks for the first 4 weeks and then every 4 weeks throughout the study. Routine liver function tests, complete blood counts and coagulation tests were determined every 4 weeks. HBV-related serological markers, including hepatitis B e antigen (HBeAg) and antibody to hepatitis B e antigen (anti-HBe), and serum levels of HBV-DNA were determined every 4 weeks during the treatment. The treatment with lamivudine was continued throughout this study. In all patients, the lamivudine-resistant HBV emerged and the elevation of serum ALT level was observed during the treatment with lamivudine. No patients were positive for antibody to hepatitis C virus (anti-HCV) and were diagnosed as having autoimmune hepatitis or drug-induced liver injury at the initiation of the treatment with lamivudine. The endpoint of this study was the last observation of patients without antiviral agents in addition to lamivudine. In patients who were treated with additional antiviral agents, such as IFN or adefovir dipivoxil, the endpoint of this study was the observation when such additional treatment started.

Serological Markers and HBV-DNA

HBeAg and anti-HBe were tested with commercial assay kits (Abbott Laboratories, North Chicago, IL). Serum HBV-DNA was quantified with commercial assay kits (Roche Amplicor Monitor polymerase chain reaction). The detection range was 2.6–7.6 log copies (LC)/ml ($10^{2.6}$–$10^{7.6}$ copies/ml). In statistical analysis, more than 7.6 LC/ml was calculated as 7.7 LC/ml. The commercially available kit, the SMITEST HBV-YMDD motif ELMA (Sumitomo Metal Industries, Tokyo, Japan), was used according to the manufacturer's instructions in order to detect the lamivudine-resistant HBV strains. The principle of this procedure is a combination of the PCR–ELISA and mini-sequence methods [23].

Classification by Serum Levels of Total Bilirubin and Prothrombin Activity

Patients enrolled in this study were placed in two categories according to the following criteria. A patient without an elevation in the serum level of total bilirubin to more than 1.5 mg/dl, but without a decline in prothrombin activity to less than 60% after the reactivation of hepatitis caused by lamivudine-resistant HBV, was defined as a patient without the potential for developing hepatic failure. On the other hand, a patient with an elevation in the serum level of total bilirubin to more than 1.5 mg/dl, and/or with a decline in prothrombin activity to less than 60% after the reactivation of hepatitis caused by lamivudine-resistant HBV, was defined as a patient with the potential for progression to hepatic failure. In our institution, the commercially available reagents, Thromborel® S (Dade Behring, Marburg, Germany) and Iatro LQ T-BIL (Mitsubishi Kagaku Iatron, Tokyo, Japan), are used for the examination of prothrombin activity and serum level of total bilirubin. The lower limit of the normal range of the prothrombin activity is 60% and the upper limit of the normal range of the total bilirubin level is 1.5 mg/dl. We used these two values of each parameter for the division of the two groups for two reasons. One reason was that we had no experiences that reactive hepatitis of patients whose prothrombin and bilirubin