Hepatitis B Virus Variants in Patients with Acute Hepatitis in whom Various Clinical Forms Develop

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Abstract

Ten patients who suffered from acute hepatitis with various clinical forms due to hepatitis B virus (HBV) were studied. HBV variants with a mutation in the precore region were dominant in two patients with fulminant hepatitis and in a patient with the most severe acute hepatitis. However, these mutant viruses were not detected in a patient who had the fulminant form of acute HBV infection on chronic liver damage or in most patients who had severe acute hepatitis. Furthermore, mutant viruses were also not detected in a patient with complicating myopathy and in one who had an atypical clinical course with three transaminase peaks. These results suggest that precore mutants may be involved in the pathogenesis of some cases of severe acute hepatitis, the same as for fulminant hepatitis, but not in other clinical forms of acute hepatitis.

Introduction

A mutation in the precore region of hepatitis B virus (HBV) DNA is closely related to seroconversion of hepatitis B e antigen (HBeAg) in carriers of HBV^{1,2)}. Furthermore, the relationship between a mutation in the precore region of HBV DNA and a fulminant change has attracted a great deal of attention in patients with acute hepatitis due to HBV^{3)~5)}. However, there have been few reports about the relationship between the mutation in the precore region of HBV DNA and other clinical forms, such as in patients with severe acute hepatitis, with the complicated form or with multiple transaminase peaks.

We treated 10 patients who suffered from acute hepatitis due to HBV which had various clinical forms including fulminant hepatitis that differed from those of typical cases. We studied HBV markers in their sera and analyzed the precore region of HBV DNA in those patients.

Subjects and Methods

Subjects

We chose 10 patients with atypical course from patients with acute hepatitis due to HBV who had been admitted to Jikei University Kashiwa Hospital (Table 1). The patients included nine men

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Table 1 Age, sex, clinical forms, laboratory data and outcome in patients with acute hepatitis due to HBV infection

Patient	Age	Sex	Clinical form	ALT (mU/ml)	HPT (%)	Outcome
1	21	F	Fulminant	8870	<10	Alive
2	66	M	Fulminant	1760	22	Dead
3	43	M	Acute on Chronic	1960	<10	Dead
4	47	M	Severe	6721	<10	Alive
5	55	M	Severe	4040	29	Alive
6	26	M	Severe	2066	31	Alive
7	25	M	Severe	1929	33	Alive
8	23	M	Severe	2576	37	Alive
9	58	M	With myopathy	1745	77	Alive
10	47	M	With 3 ALT peaks	1612	73	Alive

ALT, alanine aminotransferase; *HPT*, hepaplastin test

and one woman ranging in age from 21 to 66 years. All patients had a serum alanine aminotransferase (ALT) level of more than 1600 mU/ml. Two patients had fulminant hepatitis. An additional patient with the fulminant form of acute HBV infection had chronic liver damage due to alcohol. In these three patients prothrombin activity had decreased to less than 40% and hepatic encephalopathy which was greater than grade II had developed within 8 weeks from the onset of hepatic symptoms. Two of these patients died of hepatic failure. In the five patients who had severe acute hepatitis, the results of the hepaplastin test were less than 40%. One patient was complicated by myopathy, and another patient had an atypical clinical course in which serum levels of transaminase peaked three times.

Assay for markers of hepatitis viruses

The presence of hepatitis B surface antigen (HBsAg) and the antibody against HBs (anti-HBs) was determined by radioimmunoassay (RIA) or enzyme immunoassay, and HBeAg, the antibody against HBe (anti-HBe), and the IgM antibody to hepatitis B core antigen (IgM anti-HBc) were determined by RIA. The values of HBsAg, HBeAg and IgM anti-HBc were indicated as cut-off indeices, and that of anti-HBe as percent inhibition. DNA polymerase was assayed by means of incorporation of radioactive thymidine into DNA, and its value was indicated by cpm⁶⁾. The IgM antibody to hepatitis A virus (IgM anti-HA) was determined by RIA, and the antibody to hepatitis C virus (anti-HCV) was assayed with a second-generation HCV enzyme immunoassay kit (Dainabot Co., Ltd., Tokyo). HCV RNA was detected with a two-stage polymerase chain reaction (PCR) with two pairs of primers from the 5'-noncoding region⁷⁾.

Analysis of the precore region of HBV DNA by PCR

A mutant virus with a point mutation that changes codon 28 (TGG) of the precore region to a stop codon (TAG) was detected by restriction fragment length polymorphism (RFLP). A primer at the 5' end was designed as a mismatch primer between nucleotide positions 1875 and 1895 (5' AAGCTGTGC-CTTGGGTGGCCTT3') to amplify the HBV DNA sensitive to digestion with Bsu36 I in mutant virus (Smi Test, Institute of Immunology, Co., Ltd., Tokyo)⁸⁾.

Results

Results of HBV markers including analysis of the precore region

HBsAg was positive in all except one of the patients and IgM anti-HBc was positive in all 10. On the basis of the presence of HBeAg and anti-HBe, patients were divided into three groups on admission. In five patients HBeAg was positive and anti-HBe was negative. In three patients both

Table 2 Markers for HBV infection and analysis of the precore region of HBV DNA by RFLP in acute hepatitis patients

Patient	HBsAg	HBeAg	Anti-HBe	IgManti-HBc	Precore
1	+	±	—	+	Mutant
2	+	—	±	+	Mutant
3	+	±	±	+	Wild
4	—	—	+	+	Mutant
5	+	+	+	+	Wild
6	+	+	—	+	Wild
7	+	+	±	+	Wild
8	+	+	—	+	Wild
9	+	+	—	+	Wild
10	+	+	—	+	Wild

Fig. 1 Analysis of a point mutation in the precore region by RFLP in sera of four patients in the acute stage of hepatitis (cases 1, 2, 4, and 5) and after interferon treatment (case 1). Arrow *N* indicates an undigested band of 99 bp, and arrow *D-1* and *D-2* indicate digested bands of 79–80 bp and 19–20 bp after treatment with Sty I (*S*) or Bsu36 I (*B*). *M*, size marker; 1, 1', 2, 4, 5, case number.

HBeAg and anti-HBe were positive and in two patients HBeAg was negative and anti-HBe was positive (Table 2).

Bands digested by Bsu36 I were detected by RFLP (Fig. 1): the mutant pattern was detected in patients with fulminant hepatitis (cases 1 and 2) and in a patient with the most severe acute hepatitis (case 4). Bands after digestion by Sty I, indicating a wild-type pattern, were detected in the other cases (Table 2). A mixed pattern of mutant and wild-type viruses was not observed.

Clinical course and HBV markers in three cases

The patient in case 1 was a 21-year-old woman. She had fulminant hepatitis complicated by acute renal failure. The serum level of ALT had increased to 8870 mU/ml, prothrombin time had decreased to 12%, and hepatic encephalopathy was grade III. She recovered with intensive treatment, such as plasma exchange and interferon therapy. At the time of admission HBsAg (8.2), IgM anti-HBc (9.0), and HBeAg (2.0) were positive, and DNA polymerase (16) and anti-HBe (17) were negative. Analysis of the precore region of HBV DNA by RFLP revealed a mutant pattern, and the detected bands

Fig. 2 Clinical course of a patient with fulminant hepatitis complicated by acute renal failure (case 1). Analysis of the precore (pre-C) region of HBV DNA by RFLP revealed a mutant pattern. *IFN*, interferon; *PF*, plasma exchange; *Cr*, creatinine; *PT*, prothrombin time; *T. Bil*, total bilirubin; *ALT*, alanine aminotransferase; *C.I.*, cut-off index.

disappeared soon after interferon treatment started. IgM anti-HA, anti-HCV, and HCV-RNA were negative (Figs. 1 and 2).

In case 9 the patient was a 58-year-old man. From the onset of hepatitis this case was complicated by myopathy with myalgia and muscle weakness. By electromyography, low-amplitude waves were observed, mainly in the upper extremities. In the acute stage of hepatitis, HBsAg (42), IgM anti-HBc (7.5), and HBeAg (83) were positive, and anti-HBe was negative (0). Analysis of the precore region of HBV DNA by RFLP revealed a wild-type pattern (Fig. 3).

Fig. 3 Clinical courses of a patient with acute hepatitis complicated by myopathy (case 9) and a patient with acute hepatitis having three transaminase peaks (case 10). Analysis of the precore (pre-C) region of HBV DNA by RFLP revealed a wild-type pattern in both patients. ALT, alanine aminotransferase; T. Bil, total bilirubin; C.I., cut-off index.

The patient in case 10 was a 47-year-old man. The clinical course was atypical in that the serum level of transaminase peaked three times (1395, 1364, and 1612 mU/ml) in more than 4 weeks. During the first transaminase peak, HBsAg (59), IgM anti-HBc (6.5), HBeAg (47), and DNA polymerase (153) were positive, and anti-HBe was negative (0). During the third transaminase peak HBeAg was still

positive (60), but HBeAg (0.3) and DNA polymerase (12) became negative, and anti-HBe became positive (81). Analysis of the precore region of HBV DNA by RFLP did not reveal a mutant pattern. Neither anti-HCV nor HCV RNA was detected (Fig. 3).

Discussion

A point mutation that changes codon 28 (TGG) of the precore region of HBV DNA to a stop codon (TAG) can prevent formation of HBeAg in patients with HBV infection^{1,2)}. In addition, the mutation has been found with high frequency in patients with fulminant hepatitis due to HBV, but has not been found in patients with acute hepatitis due to HBV with a typical clinical course^{3)~5)}. However, in some studies the mutation was found with low frequency in patients with fulminant hepatitis due to HBV, and coinfection with hepatitis D virus or HCV was found in some of those patients^{9,10)}. While, there have few reports of acute hepatitis due to HBV in regard to the mutation which led to various clinical forms that differed from typical cases except for the fulminant change¹¹⁾. In the present study, we examined HBV markers in sera and analyzed the precore region of HBV DNA in 10 such patients, including patients with fulminant hepatitis.

In two patients with fulminant hepatitis HBV variants with a mutation of the precore region were predominant, as in previous reports. In case 1 HBeAg was positive but very low (2.0) at the time of admission. This finding suggests that the wild-type HBV replicated. However, massive host immunity may have caused hepatic necrosis in response to the precore mutants, because an obvious mutant pattern was detected by RFLP. Similar mutants were detected during a relapse of hepatitis after chemotherapy in an HBV carrier^{12,13)}. However, wild-type HBV replicated as the dominant virus in case 3. In this patient, the clinical course and laboratory data were believed to be similar to those for fulminant hepatitis rather than for typical acute hepatitis, as acute HBV infection was caused in a patient with chronic liver damage due to alcohol.

The most serious case of severe acute hepatitis (case 4) was not diagnosed as fulminant hepatitis, because hepatic encephalopathy was only grade I and the patient recovered without intensive treatment. On admission, HBeAg was negative, anti-HBe was positive, and a mutant pattern was observed by RFLP analysis of the precore region of the HBV DNA. This finding suggests that HBV variants do not always cause acute hepatitis to become fulminant hepatitis. In cases of severe acute hepatitis (cases 5, 6, 7, and 8) in which results of the hepaplastin test were less than 40%, wild-type HBV was the dominant virus. The point mutation that changes codon 28 (TGG) of the precore region to a stop codon (TAG) was also detected in case 4 by direct sequencing of the amplified PCR product of the entire precore region (data not shown).

Complications of the nervous system, such as myopathy, occasionally arise in patients suffering from acute hepatitis due to HBV. However, the mechanism of such complications is still unknown. In our case (case 9) we analyzed the precore region of HBV DNA, but variant HBV did not replicate as the dominant virus. In addition, cases of acute hepatitis due to HBV with multiple transaminase peaks are also occasionally encountered. In our case (case 10) we investigated the genesis of this form, but mutant HBV and HCV were not found.

These results suggest that fulminant hepatitis and severe acute hepatitis occur as a result of replication of mutant HBV. One possible reason why HBV mutants cause fulminant hepatitis and severe acute hepatitis is that HBV strains with a precore mutation are highly infectious or replicative and have numerous additional mutations^{14,15)}. Furthermore, it has been suggested that the mutation of the precore region is only a minor factor; mutations of the core-promotor or core gene might be more important in the pathogenesis of severe hepatitis^{16,17)}. Further studies are awaited to clarify the mechanisms by which HBV mutants cause fulminant hepatitis and severe acute hepatitis.

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References

- 1) Carmen WF, Jacyna MR, Hadziyannis P *et al.*: Mutation preventing formation of hepatitis B e antigen in patients with chronic hepatitis B infection. *Lancet* 2 1989; 588-591.
- 2) Akahane Y, Yamanaka T, Suzuki H *et al.*: Chronic active hepatitis with hepatitis B virus DNA and antibody against e antigen in the serum. *Gastroenterology* 1990; 1113-1119.
- 3) Kosaka Y, Takase K, Kojima M *et al.*: Fulminant hepatitis B: induced by hepatitis B virus mutants defective in the precore region and incapable of encoding e antigen. *Gastroenterology* 1991; 100: 1087-1094.
- 4) Omata M, Ehata T, Yokosuka O *et al.*: Mutation in the precore region of hepatitis B virus DNA in patients with fulminant and severe hepatitis B. *N Engl J Med* 1991; 324: 1699-1704.
- 5) Liang TJ, Hasegawa K, Rimon N *et al.*: A hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *N Engl J Med* 1991; 324: 1705-1709.
- 6) Kaplan PM, Greenman RL, Gerin JL *et al.*: DNA polymerase associated with human hepatitis B antigen. *J Virol* 1973; 12: 995-1005.
- 7) Okamoto H, Okada S, Sugiyama Y *et al.*: Detection of hepatitis C virus RNA by a two-stage polymerase chain reaction with two pairs of primers deduced from the 5'-noncoding region. *Jpn J Exp Med* 1990; 60: 215-222.
- 8) Friedman KJ, Highsmith WE Jr, Silverman LM: Detecting multiple cystic fibrosis mutations by polymerase chain reaction-mediated site-directed mutagenesis. *Clin Chem* 1991; 37: 753-755.
- 9) Feray C, Gigou M, Samuel D *et al.*: Low prevalence of precore mutations in hepatitis B virus DNA in fulminant hepatitis type B in France. *J Hepatol* 1993; 18: 119-122.
- 10) Laskus T, Persing DH, Nowicki MJ *et al.*: Nucleotide sequence analysis of the precore region in patients with fulminant analysis of the precore region in patients with fulminant hepatitis B in the United States. *Gastroenterology* 1993; 105: 1173-1178.
- 11) Takase K, Oohashi Y, Kihira T *et al.*: Late onset hepatic failure due to hepatitis B virus with mutations in the precore region. *J Gastroenterology* 1995; 30: 672-676.
- 12) Yoshida M, Sekiyama K, Sugata F *et al.*: Reactivation of precore mutant hepatitis B virus leading to fulminant hepatic failure hepatitis B virus leading to fulminant hepatic failure following cytotoxic treatment. *Dig Dis Sci* 1992; 37: 1253-1259.
- 13) Fujise K, Naito Y, Nakamura M *et al.*: Hepatitis B virus variants in carriers with hematologic malignancies in whom fulminant hepatic failure develops after chemotherapy. *Int Hepatol Commun* 1995; 4: 142-148.
- 14) Ogata N, Roger HM, Ishak K *et al.*: The complete nucleotide sequence of a precore mutant of hepatitis B virus implicated in fulminant hepatitis and its biological characterization in chimpanzees. *Virology* 1993; 194: 263-276.
- 15) Hasegawa K, Huang J, Rogers SA *et al.*: Enhanced replication of a hepatitis B virus mutant associated with an epidemic of fulminant hepatitis. *J Virol* 1994; 68: 1651-1659.
- 16) Ehata T, Omata M, Chuang WL *et al.*: Mutations in core nucleotide sequence of hepatitis B virus correlate with fulminant and severe hepatitis. *J Clin Invest* 1993; 91: 1206-1213.
- 17) Sato S, Suzuki K, Akahane Y *et al.*: Hepatitis B virus strains with mutations in the core promoter in patients with fulminant hepatitis. *Ann Intern Med* 1995; 122: 241-248.

B 型急性肝炎各種病型における変異ウイルスの関与

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要 旨

各種病型を呈した B 型急性肝炎 10 症例を対象とした。劇症肝炎の 2 例と重症肝炎の中で最重症の 1 例においては、プレコア領域に変異をもつ B 型肝炎ウイルスが主体であった。しかし、慢性肝障害に B 型肝炎ウイルスが重感染し劇症化した 1 例と大部分の重症肝炎の症例においては、プレ

コア変異株は検出されなかった。さらに、筋症を合併した 1 例とトランスアミナーゼが三峰性の経過を辿った 1 例においても、プレコア変異株は検出されなかった。これらの結果は、プレコア変異株が劇症肝炎と同様に一部の重症肝炎の病因に関与しているが、他の病型の病因には関与していない可能性を示唆している。