Non-response to Previous Interferon Therapy and Cirrhosis Are Risk Factors for Predicting Breakthrough during Lamivudine Therapy in Patients with Chronic Hepatitis B

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Aims: Lamivudine is a potent oral anti-viral medicine for the treatment of hepatitis B virus (HBV) infection. However, one of the major problems is the breakthrough (BT) followed by flare-up of hepatitis. We examined the influences of clinical background, progression of liver fibrosis, presence or absence of HBeAg and previous interferon (IFN) therapy on the occurrence of breakthrough.
Subjects and Methods: This study comprised 51 patients with HBV related chronic hepatitis (CH) or cirrhosis (LC) who were treated with lamivudine for the mean period of 33.8 ± 13.1 months (range 3–63 months). Thirty-six patients were CH, 25 were HBeAg- positive, and 25 had a previous history of IFN therapy. Patients were divided into two groups according to the occurrence of BT, either BT(+) or BT(–). Age, gender, alanine aminotransferase (ALT) and HBV titer before treatment, normalization of ALT (≤ 40 IU/L) and flare-up of hepatitis (ALT > 80 IU/L) rates, degree of hepatic fibrosis (CH/LC), presence or absence of HBeAg (HBeAg(+)/(–)), and previous IFN therapy (IFN(+)/(–)) were analyzed using Cox's proportional hazards analysis.
Results: Twenty-five patients showed BT. Background data were not different between the patients with and without BT. Flare-up of hepatitis occurred more frequently in BT. Rates of BT were markedly higher in LC (P = 0.025) and IFN(+) (P = 0.036), but HBeAg was not associated with BT. In multivariate analysis, progression of liver fibrosis (P = 0.006) and previous IFN therapy were independent risk factors for BT (P = 0.023).
Conclusions: BT significantly occurred in patients with LC and the history of previous IFN therapy. Multivariate analysis showed that progression of hepatic fibrosis and previous interferon therapy are independent risk factors for BT.

Key words: HBV, lamivudine, breakthrough

INTRODUCTION

Lamivudine is an oral anti-viral medicine for the treatment of hepatitis B virus (HBV) infection [1]. This drug was approved for the treatment of HBV-induced hepatitis [2] in November 2001, and for liver cirrhosis (LC) [3] in September 2005 by the Ministry of Health, Labor and Welfare of Japan, and is currently covered by the National Health Insurance system in Japan. The beneficial effects of lamivudine have been shown in HBV infected patients with chronic hepatitis (CH), LC and severe acute hepatitis [4]. However, one major problem is flare-up of hepatitis associated with the YMDD mutation [5, 6], occurring in approximately 50% of patients during 2 years of treatment [7]. This phenomenon is referred to as "breakthrough hepatitis" and finally leads to poor therapeutic outcome [8]. Several studies have indicated that the response to lamivudine therapy is influenced by viral load before treatment, aspartate aminotransferase (AST) level, and the presence of hepatitis Be antigen (HBeAg) [9, 10]. It was reported previously that the presence of HBeAg is an obvious risk for non-response to lamivudine therapy for HBV infected patients with chronic hepatitis and cirrhosis, by using a multivariate analysis [11]. Although this study was useful for identifying predictors for the response to lamivudine, the outcome is likely to be influenced by flare-up of hepatitis followed by virological response. Furthermore, the relation between previous IFN therapy and flare-up to lamivudine is completely unknown. This study sought to identify factors for predicting flare-up of hepatitis.

SUBJECTS AND METHODS

1. Patients

The study group comprised 51 patients with serologically proven HBV infection, and was given lamivudine at a dose of 100 or 150 mg daily. Progression of liver fibrosis especially to LC was diagnosed by histological observation with the finding of fibrotic change, or the results of ultrasound and blood tests: 1) uneven surface of the liver observed under ultrasound, 2) decrease of platelet count (plt < 10×10^4), or 3) impaired coagulation function (PT% < 80%). There

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was no patient with hepatocellular carcinoma before the treatment. The study group included 43 males and 8 females. The mean ± standard deviation (SD) age was 46.8 ± 12.0 years old. The mean follow-up period was 33.8 ± 13.1 months (range 3–63 months). There were 36 patients with CH, and 15 had LC. HBeAg was positive in 25 patients and negative in 26. All of the 25 patients who had previously received IFN therapy were non-responders, for whom the type, dosage, schedule and period varied. The other 26 patients had not previously received IFN therapy. Lamivudine was the first-choice drug at around that time; it is convenient because it is taken orally once a day and so compliance is good, and it has the advantage of normalizing the liver even though efficacy varies individually. Furthermore, non-responders to previously received IFN therapy cannot choose any other second treatment. For these reasons, most previous IFN non-responders with liver dysfunction received lamivudine therapy. The mean serum ALT levels before treatment were 209.9 ± 180.7 IU/L. Serum levels of HBV titer were expressed by transcription-mediated amplification (TMA) and hybridization protection assay [12]. The mean HBV titer before treatment was 7.1 ± 1.1 LGE/ml.

2. Study groups

Viral disappearance of HBV in serum was defined as a HBV-TMA value of less than 3.7 LGE/ml. BT was defined as an increase in viral titer to 3.8 LGE/ml or more after viral disappearance and reemergence. Patients were categorized into two groups according to the occurrence of BT:

BT positive group (BT(+)): Patients who showed BT during the observation period

BT negative group (BT(–)): Patients in whom viral disappearance persisted during the follow-up period

3. Variables in BT(+) and BT(–)

Age, gender, ALT and HBV titers before treatment and during the follow-up period in responders were compared.

4. Normalization and flare-up rates of hepatitis

Patients whose ALT level returned to the normal range (< 40 IU/L) and remained there for more than 2 months were considered as "normalized". Patients whose serum ALT level subsequently increased to more than double the upper limit (> 80 IU/L) were considered as "flare-up". The rates of normalization and flare-up were compared in BT(+) and BT(–) patients.

5. Comparison of rates

a) Influence of hepatic fibrosis

The influence of progression of hepatic fibrosis on the occurrence of BT was studied by comparing the BT rates in patients with CH and those with LC.

b) Influence of the presence or absence of HBeAg before treatment

Based on the determination of HBeAg by EIA or RIA, the influence of the presence or absence of HBeAg (HBeAg(+)/(–)) on the occurrence of BT was examined.

c) Influence of previous IFN therapy

To determine the tendency of virological tolerance in previous IFN therapy to lamivudine, the past history of IFN therapy was classified into two categories:

IFN(+): Patients who had received previous IFN therapy

IFN(–): Patients who had not received previous IFN therapy

All IFN(+) patients were non-responders, so IFN(+) means non-responder to IFN therapy too in this study. We also examined the influence of previous IFN therapy on the occurrence of BT.

6. Multivariate analysis

Cox's proportional hazards analysis was performed to determine the effect of explanatory variables (age, sex, ALT, HBV-DNA, CH/LC, HBeAg(+)/(–), and IFN(+)/(–)) on BT.

7. Data analysis

The data of backgrounds were expressed as mean ± SD. We used the Chi-squared test for comparing the rate of BT and the Wilcoxon rank-sum test for comparing continuous variables. P-values of less than 0.05 were considered statistically significant.

RESULTS

1. Clinical background characteristics

Twenty-five patients showed BT during the observation period at the mean of 36.4 months. Age, gender, and ALT levels before treatment and observation periods were not significantly different between BT(+) and BT(–) (Table 1).

2. Normalization and flare-up of hepatitis

Lamivudine normalized ALT levels in more than 95% of the patients. However, flare-up occurred much more frequently in patients with BT than in those without BT ($P < 0.001$) (Table 2). YMDD mutations were confirmed in all of 8 patients in whom YMDD mutations were assessed.

3. Comparison of the rate of occurrence of BT

a) Influence of the progression of hepatic fibrosis

The rate of BT occurrence was markedly lower in patients with CH than in those with LC ($P = 0.025$) (Table 3).

b) Influence of HBeAg

The presence or absence of HBeAg did not influence the occurrence of BT (Table 3).

c) Influence of previous IFN therapy

The rate of BT occurrence was significantly higher in patients who had previously received IFN therapy than in those who had not ($P = 0.036$) (Table 3).

4. Independent factors for the occurrence of BT using multivariate analysis

Multivariate analysis with Cox's proportional hazards model showed that the progression of hepatic fibrosis ($P = 0.006$) and previous IFN therapy ($P = 0.023$) were independently associated with BT (Table 4).

DISCUSSION

The primary aim of this study was to determine factors for predicting the success or failure of lami-

Table 1 Backgrounds

	Breakthrough (+)	Breakthrough (–)	*p*-Value
Numbers of patients	25	26	n.s.
Age	46.8 ± 12.0	42.1 ± 12.2	n.s.
Male : Female	21 : 4	22 : 4	n.s.
ALT level (IU/L)	182.3 ± 114.2	256.0 ± 226.5	n.s.
HBV-DNA levsl (LGE/ml)	7.3 ± 0.8	7.0 ± 1.3	n.s.
Observed period	36.4 ± 10.6	31.4 ± 14.4	n.s.

Table 2 Rates of normalization and flare-up of hepatitis

	Normalization		Flare-up	
	(+)	(–)	(+)	(–)
Breakthrough (+)	24	1	15	9
Breakthrough (–)	25	1	1	24
	n.s.		$p < 0.001$	

Table 3 Influences of HBeAg, liver fibrosis, previous IFN therapy on the occurrence of BT

	HBeAg		liver fibrosis		previous IFN therapy	
	(+)	(–)	(+)	(–)	(+)	(–)
Breakthrough (+)	11	14	11	14	16	9
Breakthrough (–)	14	12	4	22	9	17
	n.s.		$p = 0.025$		$p = 0.036$	

Table 4 Multivariable analysis of progression of hepatic fibrosis and previous IFN therapy using Cox's proportional hazards model

Category	Hazard ratio	95% Confidence interval (CI)	*p*-Value(Wilcoxin)
CH/LC			
CH (0)	1		
LC (1)	0.523	0.331–0.826	$p = 0.006$
IFN (+)/(–)			
(–) (0)	1		
(+) (1)	0.598	0.370–0.933	$p = 0.023$

vudine therapy. Because breakthrough hepatitis is the most frequent and major problem for unsuccessful outcome of this therapy, we used BT as the end point. Flare-up occurred significantly more often in patients with BT than in responders of lamivudine ($P < 0.001$). Among the 15 patients with flare-up of hepatitis in the BT(+) group, YMDD mutations were detected in all 8 patients tested, and all of them with flare-up showed reappearance of the virus, indicating a strong relation between the viral proliferation associated with YMDD mutations and the flare-up of hepatitis as described by Chayama *et al*. [7, 8]. One subject showed flare-up of hepatitis without breakthrough. Although the reason is not clear, his body weight increased 4 kg per year during the observation period, indicating the development of fatty liver.

BT more frequently occurred in patients with LC and with previous IFN therapy than in those with CH and without previous IFN therapy. Cox's regression analysis revealed that fibrosis of the liver and previous IFN therapy were independent risk factors for BT. Many studies have demonstrated that lamivudine is effective for the treatment of LC [3]. Lamivudine is especially beneficial in LC patients with low platelet count. However, some reports demonstrated by multivariate analysis that histological activity index is an important predictor of HBeAg loss in response to lamivudine in patients with HBV-infected CH [10, 13, 14], which would appear to support our findings.

In our study, the frequency of appearance of resistant strains was similar in HBeAg(+) and HBeAg(–) patients, indicating that the presence or absence of HBeAg is not associated with BT. Tassopoulos *et al*. found that lamivudine was effective in HBeAg(–) patients, approximating the response in HBeAg(+) patients [15]. Their result supports our findings.

Few studies have examined the relationship between previous treatment with IFN and BT. Hom *et al*. reported that previous IFN therapy did not affect the response to lamivudine. In our study, previous IFN therapy apparently influenced the response to lamivudine, Hartman *et al*. demonstrated that YMDD mutations were detected in 65% of patients who had previously received IFN therapy after one year of lamivudine treatment [16]. This rate is higher than the usual rate of appearance of YMDD mutants on lamivudine treatment, indicating that previous IFN therapy affects the response to lamivudine. We assumed that patients who had previously received IFN therapy may have an increased risk of viral resistance because of prior antiviral treatment. In our study, the IFN(+) group means non-responder to IFN therapy, too. Our results showed that non-responders to IFN therapy were less responsive to lamivudine, indicating that HBV infection may be divided into two groups with similar characteristics: good and poor responders to treatment with drugs such as IFN and lamivudine. To our knowledge, this study is the first to clearly show that previous IFN therapy and IFN non-response significantly affect BT, and are predictors for poor effectiveness of lamivudine therapy. These results will be useful for predicting therapeutic results.

CONCLUSIONS

During lamivudine treatment for HBV-infected patients with CH and LC, the rate of flare-up was significantly higher in patients with BT. The occurrence of BT was significantly higher in patients with liver cirrhosis and patients who had previously received IFN therapy. Multivariate analysis using Cox's proportional hazards model showed that the progression of hepatic fibrosis and previous IFN therapy are independent risk factors for BT.

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