Predictive factors for the response to lamivudine in HBV-infected patients with chronic hepatitis and cirrhosis

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***Objective*: To determine predictors for virological response to lamivudine, a retrospective-cohort study was designed.**
***Methods*: Seventy HBV positive patients who received lamivudine were classified according to virological response into responders and non-responders. Background conditions and normalization and flare-up of hepatitis were compared using student-t test and chi-square test. Logistic regression analysis was performed to determine the effect of explanatory variables, age, sex, ALT, HBV-DNA, hepatic fibrosisi, presence of absence of HBeAg, former IF non-response on the response to lamivudine.**
***Results*: There were no difference in gender, age, observed period, ALT level, liver fibrosis, former response to Interferon in background but viral titer and rate of HBeAg (+) was higher in non-responders. Hepatitis normalization rates were not different but flare-up rates were significantly higher in non-responders. Multivariate analysis showed HBeAg is the relevant factor for the response to lamivudine. Conclusions: The presence of HBeAg was a risk for non-response to lamivudine therapy.**

Key words: HBV, lamivudine, chronic hepatitis, liver cirrhosis, HBeAg

INTRODUCTION

Lamivudine is a potent oral anti-viral compound for the treatment of hepatitis B virus (HBV) infection [1]. This drug was approved for the treatment of chronic hepatitis (CH) in November 2001 [2] and for liver cirrhosis (LC) in September 2005 [3] in Japan. The efficacy of lamivudine has been shown not only for chronic hepatitis and cirrhosis but also for acute severe hepatitis [4]. Several studies have shown that the response to lamivudine is influenced by viral load before treatment, aspartate aminotransferase (AST) level, and the presence of hepatitis B e antigen (HBeAg) [5, 6]. However, the response to long-term treatment with lamivudine for cirrhotic patients in Japan remains poorly understood. In addition, the effectiveness of lamivudine in negative responders to former Interferon therapy remains unexplored. New antiviral drugs developed after lamivudine have already been launched. However, under the use of adefovir [7] is limited to patients who had breakthrough hepatitis during the lamivudine therapy. Therefore, lamivudine is still the key oral antiviral drug for the treatment of HBV-infected patients.

To determine predictors for virological response to lamivudine, a retrospective-cohort study was designed. We analyzed the influence of various factors such as age, gender, alanine aminotransferase (ALT), viral titer, progress liver fibrosis, presence or absence of HBeAg and previous Interferon therapy on the disappearance of serum HBV-DNA in 70 HBV-positive patients with CH and LC.

MATERIALS AND METHODS

Patients

The study group comprised 70 patients with serologically proven HBV infection, who were given lamivudine at doses of 100 or 150 mg daily at Tokai University Hospital and Tokai University Tokyo Hospital. Liver cirrhosis was diagnosed on the basis of liver biopsy or ultrasound examination as well as platelet counts and blood chemistry. Fifty-one patients of CH and 19 patients of LC with mean platelet counts of $7.7 \pm 3.1 \times 10^4$ /μl were included. There were no patients with hepatocellular carcinoma before the treatment. The study group included 55 men and 15 women. The mean age was 45.5 ± 12.2 years and their followed-up period with lamibudine therapy was 34.5 ± 13.5 months (range 3-63 months). Detection of HBeAg was performed by chemiluminescent immunoassay (CLIA) and lower than 0.1 in cut-off Index was determined as HBeAg negative. Twenty-nine patients were HBeAg-positive, and 41 were HBeAg-negative. Thirty-one patients had previously received Interferon therapy with varying type, dosage, schedule and period, and 39 had not received Interferon therapy. The mean serum ALT level before treatment was 221.4 ± 226.4 IU/L. Serum level of HBV-DNA was measured by transcription-mediated amplification and hybridization protection assay (TMA) [9]. The mean HBV titer

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Table 1 Backgrounds.

	Responders	Non-responders	*p*-Value
Numbers of patients	51	19	
Age (years)	46.8 ± 12.0	42.1 ± 12.2	n.s.
Male : Female	43 : 8	12 : 7	n.s.
ALT level (IU/L)	209.9 ± 180.7	252.3 ± 323.2	n.s.
HBV-DNA levsl (LGE/ml)	7.1 ± 1.1	7.7 ± 0.8	$p = 0.045$
Observed period (months)	33.8 ± 13.1	36.4 ± 14.6	n.s.
liver fibrosis (CH : LC)	35 : 16	16 : 3	n.s.
HBeAg ((+) : (−))	26 : 25	16 : 3	$p = 0.025$
IF ((+) : (−))	25 : 26	6 : 13	n.s.

LGE: log genome equivalent

Table 2 Rates of hepatitis normalization and flare-up.

	Normalization		Flare-up	
	(+)	(−)	(+)	(−)
Responders	49	2	16	33
Non-responders	17	2	13	4
	n.s.		$p < 0.001$	

before treatment was 7.3 ± 1.1 log genome equivalent (LGE)/ml.

Study groups

Viral disappearance in serum was defined as a negative result of HBV-TMA (<3.7 LGE/ml). Patients were categorized into two groups according to the response to lamivudine as follows: 1) Non-responders: Patients in whom HBV DNA titer was consistently positive during the follow-up period, and 2) Responders: Patients in whom viral titer became negative during the treatment.

Background conditions in responders and non-responders

To determine background conditions before lamivudine therapy, age, ALT level, HBV titer, follow-up period were compared between responders and non-responders using Student-t test. Numbers of patients in gender (Male : Female), hepatic fibrosis (CH : LC), former Interferon therapy, presence or absence of HBeAg (HBeAg (+) : (−)) in responders were compared with those in non-responders using chi-square test.

Hepatitis normalization and flare-up rates

Patients in whom ALT level settled in a normal range (≤ 40 IU/L) were categorized as "normalized". Patients in whom serum ALT level subsequently increased 2 times higher than the upper limit (80 IU/L) were categorized as "flare-up". The rates of normalization and flare-up in responders and non-responders were compared using chi-square test. The mean period for flare-up was calculated. The contribution of YMDD mutations in flare-up of responders was assessed in whom measurements were possible.

Multivariate analysis

Logistic regression analysis was performed to determine the effect of explanatory variables, such as age, sex, ALT, HBV-DNA, CH/LC, HBeAg (+)/(−), and IF (+)/(−) on the virological response to lamivudine.

Data analysis

Data were expressed in mean ± SD. Student's t-test and Chi-square test were used for background and normalization and flare-up analysis and *p*-values of less than 0.05 were considered statistically significant. In multivariate analysis, Logistic regression analysis was performed.

RESULTS

Clinical background characteristics

Fifty-one patients became negative in HBV titer measured by HBV-TMA but 19 patients persisted in positive level. Age, gender, ALT levels before treatment and observed periods were not significantly different between responders and non-responders. Serum HBV-DNA concentration before treatment was significantly lower in responders than in non-responders ($p < 0.045$). By comparing the number of patients with male or female, CH or LC, previous Interferon therapy (+) or (−) between the responders and non-responders ± were not significantly different. However, patients with HBeAg (−) were significantly well responded to lamivudine ($p = 0.025$) (Table 1).

Normalization and flare-up of hepatitis

Lamivudine well normalized ALT levels even in non-responders and the normalization rate was similar to that of responders. However, flare-ups were observed much more frequently in non-responders (Table 2) with the mean of 19.5 ± 3.1 months (range 5-40 months). Mean period for flare-ups in responders was 22.9 ± 11.4 months (range 6-51 months). YMDD mutations were confirmed in 11 responders with flare-up out of 13 patients in whom YMDD variants were assessed.

Table 3 Logistic regression analysis for determination of the effect of explanatory variables.

Factors	Hazard ratio	95% CI			*p*-value
HBV-DNA	0.679	0.334	-	1.379	0.272
ALT	1.000	0.997	-	1.002	0.741
HBeAg (+)/(−)	4.766	0.966	-	23.510	0.039
IF (+)/(−)	0.268	0.720	-	9.941	0.129
CH/LC	0.873	0.176	-	4.326	0.867
age	0.998	0.943	-	1.056	0.945
sex	3.376	0.788	-	14.460	0.096

CI: confidence interval

HBeAg (+)/(−): presence or absence of HBeAg

IF (+)/(−): previous Interferon therapy

CH/LC: progress of liver fibrosis

Multivariate analysis

Multivariate analysis performed by logistic regression analysis showed that the presence or absence of HBeAg was the relevant factor for the response to lamivudine ($p = 0.039$) (Table 3).

DISCUSSION

Lamivudine has been used for the treatment of chronic hepatitis B under the National Health Insurance system since November 2001 in Japan. Unlike Interferon, lamivudine can be used safely in patients with low platelet counts and is given orally, thereby maintaining patients' quality of life. One drawback of lamivudine that it is frequently associated with the emergence of resistant strains which accompanies a flare-up of hepatitis associated with YMDD mutations [9] is known. A new oral antiviral agent, adefovir [7], is already being used clinically. However, lamivudine must be used as the initial therapy for HBV according to the National Health Insurance system in Japan. Lamivudine thus remains the key oral medication for the treatment of HBV.

The aim of this study was to determine factors for predicting the response to lamivudine therapy. Patients were divided into responders and non-responders on the basis of their virological response. Age, gender, and AST levels before treatment were not different between responders and non-responder during similar observed periods. However, viral titer before treatment was higher in non-responders ($p = 0.045$) than responders, indicating that high viral titer negatively affected viral disappearance. Ide *et al.* reported that the viral disappearance rate with lamivudine is low (38%) in patients in whom pretreatment HBV DNA titers as measured by TMA assay are 8.0 LGE/mL or higher. Moreover, a considerable time is required for viral eradication even in patients who respond to therapy [8]. Their results are consistent with our findings. In our study, however, the normalization rates of hepatitis differed between responders and non-responders: the rates were 89.5% (17/19) in non-responders and 96.1% (49/51) in responders. Flare-up was significantly observed in non-responders (76.5%; 13/17). However, their normalized ALT persisted for 19.5 ± 3.1 months and 23.5% (4/17) showed complete suppression for approximately 3 years of their entire observed periods. This finding indicates that lamivudine is of great benefit not only for responders but also non-responders. YMDD mutations were positive in 84.6% (11/13) of responders with flare-up in whom mutations were assessed, indicating that flare-up in non-responders also are strongly associated with YMDD mutations. Flare-up occurred randomly from 5-40 months, suggesting the periodic blood tests needs to be checked at regular intervals during follow-up.

Perrillo *et al.* reported that loss of HBeAg was related to lamivudine treatment, baseline ALT level, baseline hepatitis activity index (HAI) [11], baseline HBV-DNA level, cirrhosis, and ethnic origin [6]. Their endpoint of the analysis was loss of HBeAg in serum, but in practice seroconversion is not the goal of therapy for HBV. Although our analyses and perspectives differed from theirs, our result partly supports their finding that higher HBV-DNA level negatively affected virological response. Many studies have demonstrated that lamivudine is effective for the treatment of LC [3]. In our study, progression of liver fibrosis showed no influence on virological response indicating that the use of lamivudine is truly of benefit to LC patients especially those with decreased platelet counts.

We performed multivariate analysis considering the disappearance of HBV in serum measured by TMA as a dependent variable. Logistic regression analysis revealed that the presence or absence of HBeAg affected the virological response to lamivudin. Presence or absence of HBeAg indicates presence of wild or pre-C mutation strain and the titer of HBV-DNA shows amount of virus in serum. Both of them are basically different. However, HBeAg and HBV-DNA are important because influence of those factors on therapeutic outcome were reported. Tassopoulos *et al.* found that lamivudine is also effective for HBeAg (−) patients, approximating the response in HBeAg (+) patients [11]. Our result showed that lamivudine is currently the treatment of choice for HBeAg (−) patients with chronic liver dysfunction. In our result, lamivudine is more effective for the patients with HBeAg (−) than HBeAg (+). Seroconversion of HBeAg is known to be associated with a mutation of the precore region and makes the HBV infection persist [12]. Severe hepatitis and acute chronic hepatitis are sometimes induced by this mutation. Lamivudine is not influenced by this mutation and may be efficacious for both severe acute hepatitis and persistent infection mediated by this phenomenon.

Few studies have examined the relation between

previous treatment with Interferon and response to lamivudine. In our study, previous Interferon therapy had no apparent influence on the response to lamivudine. To our knowledge, this is the first study to clearly show that previous Interferon non-response had no influence on lamivudine therapy.

Our study will hopefully provide useful information for estimating the effectiveness of lamivudine and the need for follow-up.

CONCLUSIONS

In our study, presence of HBeAg was a risk for non-response to lamivudine therapy. HBV titer before treatment was significantly higher and flare-up occurred more frequently in non-responders. However, lamivudine is real beneficial because ALT levels in the normal range persisted almost 2 years even in non-responders, and showed the same effects for LC with decreased platelet counts when comparison to CH, thus disadvantageous to the former IF non-responders were not given.

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