

• VIRAL HEPATITIS •

Lamivudine does not increase the efficacy of interferon in the treatment of mutant type chronic viral hepatitis B

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Abstract

AIM: To study the role of lamivudine in improving the efficiency of interferon for the treatment of mutant type chronic hepatitis B.

METHODS: Fifteen patients with mutant type chronic hepatitis B were prospectively studied. All patients had liver histology and serology to prove the diagnosis of chronic hepatitis B. Each patient received 4.5 million units of interferon alpha-2a thrice weekly and 100 mg of oral lamivudine daily for 24 weeks. Patients were observed and tested for blood chemistry every week for the initial 4 weeks and every 2 weeks thereafter during the treatment until 24 weeks. After the end of treatment, patients were followed up at 4-week intervals for an additional 6 months. Serum HBV DNA levels were tested using the liquid phase molecular hybridization assay. Those with non-detectable HBV DNA were also tested using the real-time polymerase chain reaction. One patient, who did not finish treatment due to depression, was excluded.

RESULTS: At the end of treatment, 7 (50 %) patients had serum ALT levels within normal limits; 12 (86 %) patients had serum HBV DNA levels <5 pg/mL using the liquid phase molecular hybridization assay, but only 8 (67%) were <20 copies/dL using the real-time polymerase chain reaction. Six months after treatment, only two (14 %) patients had a sustained complete response to the combination therapy with serum ALT level <35 IU/L and undetectable serum HBV DNA levels.

CONCLUSION: These pilot data showed that lamivudine did not increase the efficacy of interferon in the treatment of mutant type chronic hepatitis B. The liquid phase molecular hybridization assay was not sensitive enough to detect the low HBV DNA levels during combined interferon and lamivudine therapy.

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INTRODUCTION

Hepatitis B virus (HBV) is one of the major causes of liver disease worldwide^[1,2], and chronic hepatitis B can progress to cirrhosis and hepatocellular carcinoma^[1,2]. It is thus important to conduct anti-viral therapy against chronic hepatitis B to minimize the amount of liver damage^[4]. Recent studies suggest that around half of all patients with chronic HBV infection responded to a 6 to 12 month course of interferon therapy at the end of treatment with loss of serum hepatitis B viral deoxyribonucleic acid (HBV DNA) and hepatitis B e antigen (HBeAg), as well as normalization of serum alanine aminotransferase (ALT) activity^[5,6]. However, a mutant type of HBV DNA has been identified in serum from patients with chronic hepatitis B, who presented with negative HBeAg and abnormal serum ALT levels^[7]. The mutant type of HBV infection showed mutations at the precore region. Different from those patients with wild type HBV DNA, the response rate of interferon mono-therapy against mutant type chronic hepatitis B was low^[8,9]. Lamivudine has become a recent interest in the treatment of chronic viral hepatitis B^[10,11]. However, the relapse rate after the end of lamivudine therapy is high^[12-14]. Many different regimens, including a longer course of treatment or higher dosages of interferon, have been claimed to improve the interferon therapy, but the sustained response rate is still disappointing^[15,16]. Recent studies from patients with chronic hepatitis C show that the combination of interferon alpha with ribavirin results in a higher sustained response rate than interferon alpha alone^[17,18]. However, the role of combination of interferon and lamivudine against chronic hepatitis B remains uncertain^[19]. We therefore conducted the present pilot study to investigate the possible role of combined interferon and lamivudine to improve the efficiency of interferon in patients with mutant type chronic hepatitis B.

MATERIALS AND METHODS

We prospectively studied 15 documented patients with mutant type chronic hepatitis B at Cathay General Hospital, Taipei, Taiwan between June 1999 and June 2001. All patients were males aged between 20 and 65 years old (mean \pm SD: 44 \pm 8 years), and were naive without prior interferon or other anti-viral therapy. Chronic hepatitis B was defined as positive hepatitis B surface antigen (HBsAg, Auszyme, Abbott Laboratory, Abbott Park, IL 60064) and abnormal serum ALT levels (normal <35 IU/L) for more than 6 months. Mutant type chronic hepatitis B was defined as detectable HBV DNA, positive HBsAg, negative HBeAg [HBeAg; HBe (rDNA) EIA, Abbott Laboratory], and abnormal serum ALT levels in patients having chronic hepatitis B. All patients had at least three documented occasions of abnormal serum ALT levels higher than twice the upper limit of normal with one month apart, within 6 months prior to enrollment. All patients underwent liver biopsy within one month before the start of treatment to confirm the chronic hepatitis without cirrhosis.

None of our patients were alcoholic, intravenous drug users

or homosexual. None had received hepatotoxic drugs, herbal medicine or immuno-suppressive therapy within the past 6 months. Further, none had decompensated liver function (prolonged prothrombin time >3 seconds vs. INR >1.50, serum total bilirubin >3.0 mg/dL, or serum albumin < 3.0 g/dL), cirrhosis, chronic renal failure, clotting abnormalities, hemophiliacs, serious neurological disorders, obesity, chronic viral hepatitis C (Murex anti-HCV, Version III, Murex Diagnostics Ltd., Dartford, England) or delta (Wellcozyme, Wellcome Diagnostics, Dartford, UK), autoimmune disease (anti-nuclear antibody titer >1:40, Fluoro HEPANA, Medical & Biological Lab., Nagoya, Japan), and/or inheritable disorders such as hemochromatosis, alpha-1-antitrypsin deficiency or Wilson's disease. All patients with peripheral white cells <4 000 per mm³ and platelet < 100 000 per mm³ were excluded. Serum HBV DNA was measured using a commercially available liquid phase molecular hybridization assay (Digene Hybrid Capture™ System, Beltsville, MD) according to the manufacturer's instruction. The lowest detectable HBV DNA level was 5 pg/ml. Serum samples with non-detectable HBV DNA were retrospectively tested using the real-time polymerase chain reaction (RT-PCR) methods after the end of clinical follow-up. The lowest detectable HBV DNA level was 20 copies/mL.

All patients received 4.5 millionunits (MU) of interferon alpha-2a (Roferon, F. Hoffmann-La Roche Ltd., Basel, Switzerland) thrice weekly and 100 mg of oral lamivudine daily for 24 weeks. Patients were observed and tested for blood chemistry every week for the initial 4 weeks and every 2 weeks thereafter during the treatment until 24 weeks. After the end of treatment, patients were followed up at 4-week intervals for an additional 6 months.

Normalization of serum ALT levels and absence of serum HBV DNA were assessed for the efficacy of treatment. A complete response was defined as the normalization of serum ALT levels together with the absence of serum HBV DNA by the end of treatment. A sustained complete response was defined as the continuation of the remission for at least 6 months after the end of treatment.

Informed consent was obtained from the patients before treatment. The study protocol was reviewed and approved by the Institutional Review Board of the hospital under the guidelines of the 1975 Declaration of Helsinki.

Statistical analysis

Was performed using two-tailed Student's *t*-test and two-tailed Fisher's exact test where appropriate.

RESULTS

The demographic data, biochemical data and serum HBV DNA levels are shown in Table 1. The total bilirubin were 0.9±0.2 mg/dL (data are presented as mean ±S.D. and so forth; range: 0.4-1.8 mg/dL), and prothrombin time (INR) was 1.01±0.08 seconds (range: 0.81-1.22) before treatment. The mean serum ALT levels were 132±71 IU/L (range: 42-290 IU/L), and mean serum HBV DNA were 224±255 pg/mL (range: 6-1924 pg/mL) before treatment.

The major side effects of treatment were: flu-like symptoms: 8 (53 %), fatigue: 12 (80 %), insomnia: 4 (29 %), hair loss: 5 (33 %), and depression: 1 (7 %). None developed anemia (hemoglobin <11 g/dL), leukopenia (leukocyte <3500/μl), thrombocytopenia (thrombocytes <100×10³/μl), hyperbilirubinemia (>2.0 mg/dL), or pancreatitis during therapy.

Fourteen patients finished a 24-week-course of treatment.

One patient (HSF) discontinued interferon after 2 months of therapy due to interferon-related depression. His serum ALT remained abnormal from 210 IU/L before treatment to 38 IU/L at the end of treatment, and serum HBV DNA levels dropped from 90 pg/mL before treatment to 29 pg/mL at the end of therapy. None of the remaining 14 patients experienced depression, aggression, hostility or hallucination.

The serum ALT levels dropped from 132±71 IU/L before treatment to 46±17 IU/L at the end of treatment (Table 2, *P*=0.0002), and the serum HBV DNA levels decreased from 224±255 pg/mL before treatment to 8±12 pg/mL at the end of treatment (*P*=0.058). At the end of treatment, 7 (50 %) of the 14 patients had serum ALT levels within normal limits. Although 12 (86 %) patients had undetectable serum HBV DNA levels (<5 pg/mL) using the liquid phase molecular hybridization assay, only 8 of them had undetectable HBV DNA (<20 copies/mL) using the RT-PCR. Both of these patients had complete response with normal serum ALT levels and undetectable at the end of treatment. The remaining 12 patients had abnormal serum ALT levels and/or detectable HBV DNA.

Six months after the end of treatment, the mean serum ALT (90±58 IU/L, *P*=0.03) and HBV DNA (63±70 pg/mL, *P*=0.05) levels increased compared to those at the end of treatment. Three patients continued to have undetectable serum HBV DNA using the liquid phase molecular hybridization assay method. However, only two (14 %) of them had both complete response and sustained complete response to the combination therapy with serum ALT level <35 IU/L and undetectable serum HBV DNA levels using the RT-PCR method. One patient with undetectable HBV DNA using the liquid phase molecular hybridization assay had abnormal serum ALT levels after the end of treatment; his serum HBV DNA level was 93 copy/dL using the RT-PCR. All of the remaining 11 patients had abnormal serum ALT levels as well as detectable serum HBV DNA.

Table 1 Demographic data, biochemical data and serum HBV DNA levels of patients

Patient No	Age/Gender (y)	ALT (IU/L)	Total Bilirubin (mg/dL)	Prothrombin time (INR)	HBV DNA (pg/mL)
1*	52/M	210	0.9	1.02	90
2	65/M	42	0.9	0.93	24
3	61/M	162	1.4	1.18	1924
4	43/M	38	0.8	0.91	107
5	45/M	212	0.8	1.00	303
6	34/M	49	0.4	1.02	66
7	38/M	47	0.6	1.05	262
8	36/M	119	0.9	0.81	6
9	35/M	56	0.8	0.90	7
10	48/M	154	0.5	1.12	265
11	46/M	223	1.0	1.14	56
12	38/M	290	1.8	1.16	8
13	41/M	44	1.0	0.93	8
14	39/M	195	0.7	1.03	11
15	60/M	147	0.7	1.07	17

*Patient 1 received only 8 weeks of interferon therapy

Both of the two patients with sustained complete response were less than 40 years old (36 and 38 years old) with higher initial serum ALT levels >100 IU/dL (119 and 290 IU/dL), lower initial serum HBV DNA levels <10 pg/ml (6 and 8 pg/mL), and a histology with moderate intralobular degeneration and focal necrosis (range: 1/3-2/3 of lobules). Using the two-tailed Fisher's exact test, age <40 year-old ($P=0.11$), serum ALT level >100 IU/dL ($P=0.37$), serum HBV DNA < 10 pg/ml ($P=0.07$), and moderate to severe intralobular degeneration and focal necrosis ($P=0.07$) were not significant factors in predicting sustained complete response.

Table 2 Serum ALT and HBV DNA levels of patients before, at the end, and at 6 months after treatment

Patients	ALT (iu/L)			HBV DNA			
	start	end	6M	start	end	6M	
					LPMH PCR	LPMH PCR	
1 ^a	210	38	51	90	29	-	-
2	42	97	166	24	18	-	30
3	162	57	84	1924	ND	24.4	13
4	38	34	37	107	ND	78.8	7
5	212	26	66	303	ND	5.1	27
6	49	46	150	66	63	-	98
7	47	35	43	262	ND	94.7	27
8	119	24	20	6	ND	ND	ND
9	56	80	41	7	ND	43.8	ND
10	154	54	254	265	ND	1.3	128
11	223	48	215	56	ND	1.2	445
12	290	30	24	8	ND	ND	ND
13	44	22	71	8	ND	9.7	10
14	195	56	41	11	ND	2.2	39
15	147	22	47	17	ND	12.6	10
Mean	132	46 ^b	90 ^c	224	8 ^d		63 ^c
S.D.	71	17	58	255	12		70

ND: HBV DNA <5 pg/ml using the liquid phase molecular hybridization assay (LPMH); HBV DNA < 20 copies/dL using the real-time polymerase chain reaction (PCR). ^aPatient 1 received only 8 weeks of interferon therapy; ^b $P=0.0002$, ^d $P=0.05$ compared with those before treatment; ^c $P=0.058$, ^e $P=0.05$ compared with those at the end of treatment

DISCUSSION

At the end of treatment, the mean serum ALT level was decreased, and half of the patients had a serum ALT level within normal limits. Although most patients had undetectable HBV DNA levels using the liquid phase molecular hybridization assay, the change of serum HBV DNA levels was not statistically significant, a finding consistent with studies from patients of chronic viral hepatitis B with wild type HBV DNA^[11,20].

Six months after the end of treatment, the mean serum ALT level was significantly increased compared with those at the end of treatment. Only 14 % of patients had sustained response with normal serum ALT levels as well as undetectable HBV

DNA levels. The low sustained response rate to combined interferon alpha-2a and lamivudine was consistent with those of interferon mono-therapy for patients with mutant type chronic hepatitis B^[8,9]. Our pilot data showed that lamivudine did not increase the efficacy of interferon in the treatment of mutant type chronic hepatitis B, a finding consistent with studies stating those having positive HBeAg that IFN and lamivudine did not offer additional benefit compared with lamivudine monotherapy^[20].

Although 12 of the 14 patients (86 %) had an undetectable HBV DNA level at the end of treatment using the liquid phase molecular hybridization assay, half of them had abnormal serum ALT levels. Only 14 % and 21 % of the 12 patients with undetectable HBV DNA levels using the liquid phase molecular hybridization assay had normal serum ALT levels and undetectable serum HBV DNA using the RT-PCR method. RT-PCR is much more sensitive than liquid phase molecular hybridization assay in the detection of samples with low HBV DNA levels.

For the 15 serum samples with undetectable HBV DNA using the liquid phase molecular hybridization assay, only 10 of them had an undetectable HBV DNA level using the RT-PCR. These four samples were from eight and two patients at the end and 6 months after treatment. Both of these patients had sustained normal serum ALT levels and undetectable serum HBV DNA at 6 months after treatment. For the remaining 5 patients, low titer HBV DNA was identified using the RT-PCR, and all patients developed abnormal serum levels after the end of treatment. Our data showed that the liquid phase molecular hybridization assay was not sensitive enough to detect the low HBV DNA levels during combined interferon alpha-2a and lamivudine therapy. RT-PCR method is better than liquid phase molecular hybridization assay in the detection of low titer HBV DNA. The presentation of low levels of HBV DNA may result in subsequent abnormal serum ALT levels and increasing HBV DNA levels in 6 months.

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