Lamivudine monotherapy and lamivudine plus interferon alpha combination therapy in HBeAg negative chronic hepatitis B not responding to previous interferon alpha monotherapy

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Abstract

Background and study aims : **To investigate the efficacy of the combined therapy of lamivudine (LAM) plus alpha interferon (IFN) and LAM monotherapy in HBeAg negative chronic hepatitis B (CHB) patients who were unresponsive to previous IFN monotherapy, and the incidence of YMDD mutations.**

Patients-Methods : **Forty-five HBeAg negative patients were enrolled in this study. 24 of these were treated with LAM (100 mg/day, PO, for 24 months) alone (group 1) and 21 with combined therapy (IFN-alpha-2b, 10 MU, tiw, SC, for 6 months plus LAM 100 mg/day, PO, for 24 months) (group 2). Normal alanine aminotransferase values and negativity of HBV DNA (molecular hybridization ; Digene, USA) were accepted as treatment response. YMDD variants were analyzed at the end of treatment or when clinical breakthrough was observed (Inno-Lipa Innogenetic kit, Belgium).**

Results : **End of follow-up response rate was 29.2%, by ITT in group 1, 19% in group 2 ($p > 0.05$). Histological activity index was statistically decreased by LAM monotherapy as compared to combination therapy. YMDD mutation rates were 59% in group 1, 62.5% in group 2 ($p > 0.05$).**

Conclusions : **Additional IFN-alpha therapy to LAM in HBeAg negative CHB not responding to previous IFN-alpha monotherapy does not increase the response rate compared to LAM monotherapy and does not also decrease the incidence of YMDD mutations.** (Acta gastroenterol. belg., **2007**, 70, **20-24**).

Key words : HBeAg negative chronic hepatitis B, interferon nonresponder, YMDD mutations.

Abbrevations

Hepatitis B virus (HBV), interferon (IFN), lamivudine (LAM), chronic hepatitis B (CHB), hepatitis B e antigen (HBeAg), antibody to hepatitis B e antigen (anti-HBe), hepatitis B surface antigen (HBsAg), antibody to hepatitis C virus (anti-HCV), histological activity index (HAI)

Introduction

Hepatitis B virus (HBV) infection is a major problem in the world. One quarter of chronic carriers develop evidence of chronic progressive hepatic inflammation. Half of these patients progress to cirrhosis within 5 years. In Turkey, anti-HBe positive CHB is more frequent than HBeAg positive CHB, like in other Mediterranean and Asian countries (1). Hepatitis B e antigen (HBeAg) negative CHB is different from HBeAg positive CHB in respect to the natural course and therapeutic features. Three therapeutic agents, interferon (IFN) alpha, lamivudine (LAM) and adefovir are currently approved in many countries for the treatment of chronic hepatitis B (CHB) (2). After IFN alpha therapy, relapse rate is generally high, and long term response rate was observed in only 20% to 25% of HBeAg negative CHB patients (3). On the other hand, beneficial effects of LAM in HBeAg negative CHB patients was observed after 12 months of treatment (virological response of 60%-70%) (4,5). But, relapse was high with discontinuation of LAM. The other main problem is the development of resistance during the LAM treatment (6). There is no standard therapy for HBeAg negative CHB refractory to IFN alpha monotherapy. Data on IFN monotherapy or IFN plus LAM combination re-treatment of patients with HBeAg negative CHB are very limited (7). Combination therapy theoretically may be more effective than monotherapy with either IFN alpha or LAM. We investigated the efficacy of the combined therapy of LAM plus IFN alpha and LAM monotherapy in HBeAg negative CHB patients nonresponder to previous IFN alpha monotherapy, and incidence of YMDD (tyrosine-methionine-aspartate-aspartate amino acid motif of HBV polymerase) mutations.

Patients and methods

Forty-five HBeAg negative/antibody to hepatitis B e antigen (anti-HBe) positive CHB patients that were nonresponders to previous 6 months of IFN alpha monotherapy were enrolled in this study. Eligibility criteria before treatment included : (i) Hepatitis B surface antigen (HBsAg) and anti-HBe positivity and HBeAg negativity for at least 18 months, (ii) HBV DNA (molecular hybridization technique, cut-off level : 4 pg/ml) positivity in serum for 6 months or more, (iii) elevation (at least $> 1.3 \times$ upper limit of normal) of serum alanine aminotransferase (ALT) levels for 3 months or more, (iiii) biopsy proven chronic hepatitis and compensated

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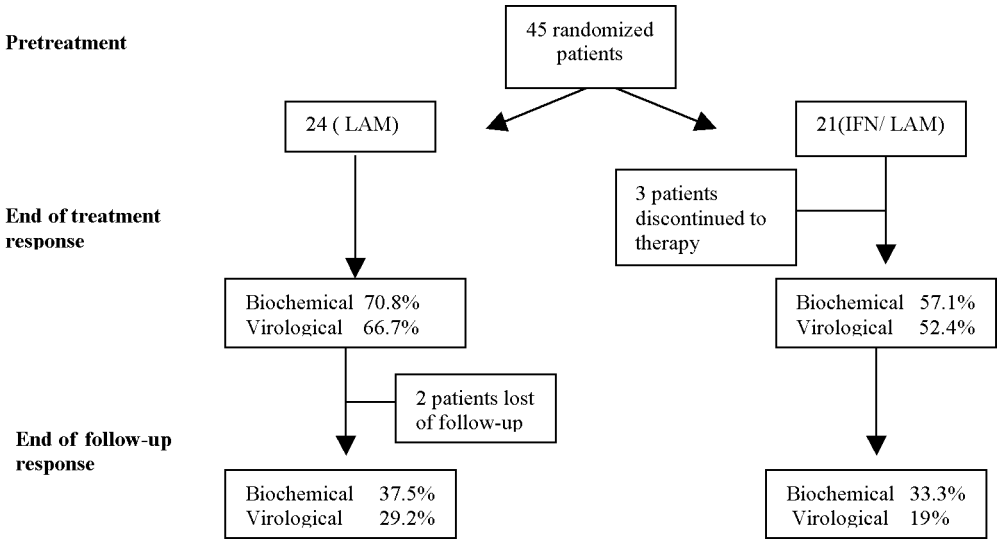

*The results are expressed as ITT
IFN : interferon, LAM : lamivudine.

Fig. 1. — The flow chart of the study

liver disease. All of the known other aetiologies of chronic hepatitis were negative in these patients. Antibody to hepatitis C virus (anti-HCV) and total antibody to hepatitis delta virus (anti-delta total) were also negative in all patients. Of the patients, 39 were primary nonresponders and the remaining 6 were relapsers. The patients were randomized for this study within 6-15 months after finishing IFN alpha monotherapy. The study protocol was approved by the local ethic committee, and informed consent was taken from all patients.

Twenty four of these were treated with LAM (100 mg/day, PO, for 24 months) alone (group 1) and 21 with combined therapy (IFN alpha 2b, 10 MU, tiw, SC, for 6 months plus LAM 100 mg/day, PO, for 24 months) (group 2). IFN alpha and LAM were started concomitantly in group 2. Patients' visits were done once a month while giving IFN alpha therapy and then every three months while giving LAM monotherapy. At each visit, routine haematological and biochemical parameters were tested. Liver biopsy was performed at baseline in all patients. At the end of treatment, biopsy was re-performed only in those who accepted this procedure. Histological changes (histological activity index (HAI), and the extent of fibrosis) were assessed according to the Knodell scoring system (8).

At baseline, end of treatment and during the clinical breakthrough, blood were drawn from all patients and centrifuged at 2500 rpm for 5 minutes and separated serum samples were stored at –85°C. HBsAg, anti-HBs, anti-HBe and HBeAg were tested by using immunoenzymatic assays (Organon Teknika (Holland) for HBsAg and anti-HBs, Pasteur (France) for anti-HBe and HBeAg). Anti-HCV and anti-HDV total were determined by UBI EIA 4.0 (Organon Teknika (Holland) and Pasteur (France)). HBV-DNA was investigated on serum samples using molecular hybridization (Digene, USA). The lowest detection limit of this assay is 4 pg/ml. YMDD variants were searched in extracted DNA from sera by BOOM (silica) method (9-11).

PCR was performed in 100 µl serum samples, DNA dissolved in 50 µl TE buffers. After this step, HBV polymerase gene region was amplified in two steps by using PCR primer with biotin (INNO-LiPA HBV DR Amplification (INNOGENETICS N.V, Belgium kit). In order to avoid contamination, a maximum physical separation between the pre- and post- amplification steps (separate rooms, separate pipettes and other lab material, separate lab coats and gloves) was done. We used positive and negative control for each groups during DNA extraction and PCR steps. All the protocols were applied for avoiding contamination as described previously (9). HBV mutations detected by INNO-LiPA HBV DR (INNOGENETICS N.V, Belgium). INNO-LiPA HBV DR is based on the reverse hybridization principle. The INNO-LiPA HBV DR strips contain wild-type and mutant-type or polymorphic HBV DR probes detecting the polymerase sequencing coding for aminoacids. Biotinylated DNA material is hybridized with specific oligonucleotide probes immobilized as parallel lines on membrane-based strips. After hybridization, streptavidin labelled with alkaline phosphatase is added and bound to any biotinylated hybrid previously formed. Incubation with BCIP/NBT chromogen results in a purple/brown precipitate. Using INNO-LiPA HBV DR, wild type and mutations or polymorphism at codons 528 (180), 552 (204) and 555 (207) of the HBV polymerase gene can be detected simultaneously. Also chances at codon positions 171, 172, 195, 196, 198, and 199 of HBsAg can be identified due to the overlapping reading frame.

Normal ALT values and negativity of HBV DNA were accepted as end-treatment response. Maintaining of this response to the end of follow-up period (at least 6 months) was accepted as end of follow-up response (EFR). Clinical breakthrough was characterized by elevation of ALT values (> 1.5 × upper limit of normal or beginning level) and the reappearance of HBV DNA in serum. All patients were followed up without treatment after the treatment period of 24 months finished. IFN alpha therapy was discontinued by only 3 patients because of adverse affects (weight loss, fever and myalgia). Two patients did not come for follow up after treatment in group 2. YMDD variants were analyzed at the end of treatment or when a clinical breakthrough was observed.

Data are presented as mean± SEM. Data analysis was made by the Chi-Square, Fisher's exact, independent t-tests and Pearson correlation tests SPSS 10.0 version for Windows, where appropriate. Per protocol (PP) analysis was done for patients who completed the treatment and follow-up periods. The intent-to-treat (ITT) analysis included data for all patients who were randomized to a study group. A value of $p < 0.05$ was considered statistically significant.

Results

Baseline characteristics of patients were similar in the two groups (Table 1). End of treatment response (ETR) rate was 66.7% by both ITT and PP analysis in group 1. This rate was 52.4% and 61.1% by ITT and PP, respectively in group 2 ($p > 0.05$). End of follow-up response (EFR) rate was 29.2% and 31.8% by ITT and PP, respectively in group 1. This rate was 19% and 22.2% by ITT and PP, respectively in group 2. Both ETR and EFR rates were higher in group 1 than in group 2, but the differences were statistically insignificant ($p > 0.05$). Age, gender, histological stage, HAI, initial ALT and HBV DNA levels had no effect on response rate by multivariate regression analysis.

Clinical breakthrough was observed in 6 patients in group 1 (two at month 12, four at month 24) and 4 in group 2 (One at month 12, three at month 24). Mean ALT and HBV DNA levels were 179.2 ± 201.4 IU/L (70-576), 507 ± 771.9 (4-2000) pg/ml in group 1, respectively ; and 91.1 ± 27.2 IU/L (70-130), 550 ± 968.3 (5.6-2000) pg/ml, in group 2, respectively, during clinical breakthrough. None of the patients had clinical findings of hepatic decompensation.

Presence of YMDD mutation was searched in 22 patients in group 1, 16 patients in group 2. YMDD mutations were detected in 13 patients (59%) from group 1 and in 10 (62.5%) from group 2 (Table 2). YMDD variants were similar in both groups. Phenotyping and genotyping resistance rates to lamivudine were comparable between the two groups ($p > 0.05$). Age, gender, therapy modality, HAI, histological stage, ALT and HBV DNA levels before treatment were similar between YMDD mutation positive and negative patients.

During the follow-up period, liver biopsy was performed in 12 patients (5 of them responders) in group 1, 7 patients (2 of them responders) in group 2 (Table 3). Statistically significant decreasing for HAI in post treatment liver biopsy specimens was observed in group 1. This decreasing was observed in both responder ($p = 0.024$) and nonresponder ($p = 0.011$) patients with LAM monotherapy.

Discussion

HBeAg negative, anti-HBe positive and HBV DNA positive form of CHB is referred as HBeAg negative CHB or precore mutant CHB (12,13). HBeAg negative CHB represents a potentially severe and progressive type of liver disease with frequent development of cirrhosis and hepatocellular carcinoma (14-15). Therefore, all patients whom HBeAg negative, anti-HBe positive and moderate to severe necroinflammation on liver histology are candidates for therapy. Currently there are

Table 1. — **Baseline characteristics of the patients**

	Group 1 (LAM)	Group 2 (IFN + LAM)
n	24	21
Age (mean, range) years	45.04 ± 8.3 (25-65)	41.4 ± 9.6 (20-60)
Female/Male	9/15	4/17
ALT (IU/L)	126.25 ± 79.3	179 ± 127.3
HBV DNA (pg/ml)	426.4 ± 549.8	785 ± 933.7
HAI	9.3 ± 4.2	10.2 ± 4.9
Histological stage 1/2/3/4 (n)	9/9/5/1	6/5/7/3

Table 2. — **YMDD variants in the study groups**

Mutations	Group 1 (n = 22)	Group 2 (n = 16)
L528M (L180M), M552V (M204V)	6	4
L528M (L180M), M552I (M204I)	4	2
M552I (M204I)	2	3
M552V (M204V)	1	1
Total	13 (59%)	10 (62.5%)

Table 3. — **Liver biopsy findings baseline and after treatment**

	Baseline	After treatment	p*		Baseline	After treatment	p*
Group 1				**Group 2**			
Total (12)				**Total (7)**			
HAI	9.7 ± 3.3	3.1 ± 2.3	< 0.05	HAI	9.7 ± 3.9	5.6 ± 3.6	> 0.05
Histological stage (1/2/3/4)	4/4/3/1	7/3/1/1		Histological stage (1/2/3/4)	1/4/2/0	5/0/2/0	
Responder (5)				**Responder (2)**			
HAI	10.2 ± 2.9	2.8 ± 2.6	< 0.05	HAI	12 ± 5.7	3 ± 4.2	> 0.05**
Histological stage (1/2/3/4)	2/1/2/0	3/1/1/0		Histological stage (1/2/3/4)	0/1/1/0	1/1/0/0	
Nonresponder (7)				**Nonresponder (5)**			
HAI	9.3 ± 3.9	3.5 ± 2.3	< 0.05	HAI	8.8 ± 3.3	6.6 ± 3.3	> 0.05
Histological stage (1/2/3/4)	2/3/1/1	4/2/1/0		Histological stage (1/2/3/4)	1/3/1/0	3/0/2/0	

* Baseline HAI versus after treatment HAI
** Nonparametric Wilcoxon Signed Ranks test.

only two drugs, LAM and IFN alpha, that are commonly used for the treatment of CHB. Adefovir is generally used for lamivudine resistant patients. Limited beneficial effects can be obtained with IFN alpha monotherapy. Even end of treatment response rate is high, sustained response rate is low (15-25%) because of frequent relapses (16-17). In our study, 45 HBeAg negative CHB patients that were nonresponders to previous IFN alpha therapy were randomized into two groups.

Limitations of our study are mainly related to the absence of a placebo control, but such a design would not be accepted in our patients due to ethnic reasons and to the fact that we compared the efficacy of LAM monotherapy and IFN alpha plus LAM combination therapy. Pegylated-IFN was not used in this study since it was only started to be used in clinical trials for the treatment of chronic hepatitis B when this protocol was begun. Now it has been approved by the Health Authority in our country. Finally, HBV phenotyping was not performed although recent literature suggests that genotype might impact outcome of treatment (18,19).

This study clearly shows that the treatment response rate was similar in LAM monotherapy and IFN alpha plus LAM combination therapy in patients not responding to previous IFN therapy. Lamivudine is well-tolerated oral nucleoside analogue for patients with CHB. In long term follow-up period sustained response rate was however less than 10% with LAM monotherapy (20). Both biochemical and virological relapses were observed in most patients after discontinuation of lamivudine therapy. Our EFR rate was slightly high (29.2% by ITT in group 1) compared with literature. If the follow up period had been longer, probably EFR rate would have decreased.

Theoretically, combination of IFN alpha and LAM might lead to a better sustained response rate than monotherapy. Sustained response rate was reported to be 14% in naïve HBeAg negative CHB patients that received combination therapy and no YMDD variant emerged in this study (21). EFR (19% by ITT) was compatible with literature in our group 2. There are limited studies about treatment of HBeAg negative CHB patients that were nonresponders to previous IFN alpha therapy (22-24). Yang *et al.* (25) showed that combination therapy with IFN alpha and LAM did not increase the efficacy of IFN alpha in the treatment of naïve HBeAg negative CHB as in our study. But, we have to mention that our patients were nonresponder to previous IFN alpha therapy. Also, in a pilot study, no beneficial effect of combination therapy was detected in CHB (26). Although LAM monotherapy was effective, combination of LAM and IFN alpha therapy was not effective in HBeAg positive CHB in IFN nonresponders (27). Post treatment liver biopsy showed significant regression for HAI in both responder and nonresponder patients in LAM monotherapy group. Dienstag *et al.* (6) also showed that three years of LAM therapy reduces necroinflammatory activity and reverses fibrosis (including cirrhosis) in most patients. We found that combination therapy was inferior to LAM monotherapy in histological response. However, this can be related with few control liver biopsy in combination group. The continuing therapy with LAM in patients treated with IFN or not may provide a chance for histological improvement.

On the other hand, continuation of LAM was associated with the development of drug resistance (2). In one study, the rate of LAM resistance was reported as 19% at the end of first year and 44% at the end of second years (28). In our study, YMDD mutation rate was 59% in group 1, 62.5% in group 2. The development of YMDD mutant was not decreased by IFN alpha plus

LAM combination therapy. However, Santantonio *et al.* (29) reported that combination regimen prevented the emergence of YMDD variants. In this study, patients had received the treatment for 12 months. While the duration of LAM treatment is getting longer, preventive or delaying effects of interferon on the development of YMDD variant may disappear.

In conclusion, additional IFN alpha therapy to LAM in HBeAg negative chronic hepatitis B not responding to previous IFN monotherapy does not increase the response rate compared to LAM monotherapy. Combination therapy does not decrease the emergence of YMDD mutations.

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