**INTRODUCTION**

Despite the use of HBV vaccine, chronic hepatitis B virus (HBV) infection occurs approximately in 5% of the global population. This infection, if persistent, may lead to chronic hepatitis, cirrhosis and hepatocellular carcinoma in 25% to 40% of infected patients and is among the principle 10 causes of death throughout the world [1, 2].

Until recently, the only generally approved treatment for chronic hepatitis B was alpha-interferon, but it has demonstrated moderate efficacy in terms of sustained response (biochemical, virological and histological). In fact, only 20% to 40% of treated patients responded to therapy, with lower percentages (~10%) among patients infected with precore-mutant strains of HBV (HBeAb HBV-DNA positive) [3, 4]. This form, prevalent in our geographic area (~90% of the all patients with HBV infection), is due to a mutation at nucleotide 1896 in the precore region of the HBV-DNA genome. The result of this mutation is a stop codon that blocks HBeAg synthesis but still permits HBV replication and hepatitis B core antigen production, leading to persistence of viremia and persistent or intermittent elevated serum alanine aminotransferase (ALT) levels with frequent evolution of disease into cirrhosis and hepatocellular carcinoma. The suboptimal response of this form to alpha-interferon with a high rate of non-responders or relapsers has led to the research and development of new antiviral drugs to be used as alternative therapies.

At the end of 1990s, a new drug, lamivudine, a negative isomer of the racemic mixture of 2-deoxy-3-thiacytidine, was shown to be a powerful inhibitor of HBV reproduction both in wild type chronic hepatitis and in the pre-core mutant form in randomized studies in Europe, North America and Asia. The optimal dose which significantly reduces the serum ALT and HBV DNA levels has been determined to be 100 mg/daily for 12 months with few side effects. However, findings in the literature and in our previous study indicate that an excellent response to treatment (80%) can result in renewal of disease activity after interruption of therapy, manifested as severe hepatitis in some cases [5-10]. Therefore, to increase the percentage of sustained response, trials using prolonged lamivu-
dine therapy or an association of α-interferon plus lamivudine have been initiated, as the two drugs work with different mechanisms of action [11-17].
The aim of this study was to assess the efficacy and tolerability of lamivudine (LAM) versus lamivudine plus α-interferon (IFN) for treatment of chronic anti-HBe positive hepatitis B in patients with precore-mutant variants.

**PATIENTS AND METHODS**

**Patients**
A total of 59 patients (28 males, 31 females) with chronic anti-HBe positive HBV infection were included in the study. All patients had been previous non-responders to 2 or 3 cycles of interferon alpha therapy; the last cycle was completed ≥6 months before starting the present study. Additional inclusion criteria were as follows:

a) serum ALT levels >2 times normal level for >6 months;

b) HBV infection based on the presence of hepatitis B surface antigen (HbsAg) in the serum, determined by the enzyme-linked immunosorbent assay ELISA (Monolisa Ag HBs, Pasteur) and HBV DNA positivity >5 pg/ml, determined by sandwich hybridization testing for nucleic acid (Quantiplex, Chiron);

c) positive histology for chronic hepatitis/cirrhosis within 6 months of the study according to the Knodell-Ishak classification (Histological Activity Index [HAI] average score ∼13).

The presence of YMDD (tyrosine-methionine-aspartate-aspartate amino acid motif of HBV polymerase) mutations were assessed at week 52. The detection of the YMDD variants was performed using a restriction fragment linked polymorphism assay.

Patients were excluded from the study if other causes of chronic hepatitis (hepatitis C virus infection, hepatitis D virus infection, autoimmunity, alcoholism, Wilson’s disease, hemochromatosis, alpha-1-antitrypsin deficiency) could not be ruled out. Additional exclusion criteria included past or present episodes of hepatic failure (eg, ascites, bleeding of esophageal varices and encephalopathy), a positive test for human immunodeficiency virus (HIV) and drug addiction.

Approval for the study was obtained from the ethics committee. Written informed consent was obtained from all patients in the study. All study procedures met the criteria of the 1975 Declaration of Helsinki and its subsequent amendments.

**Study design**
Patients were randomly divided into 3 groups:

a) 21 patients received LAM at 100 mg/daily orally for 52 weeks;

b) 20 patients received LAM at 100 mg/daily plus IFN at 6 MU subcutaneously three times weekly for 52 weeks;

c) 18 patients received the same combination therapy for 40 weeks after pre-treatment with lamivudine for 12 weeks.

The virological and biochemical response to treatment were defined as follows:

1) sustained response indicated serum ALT levels within normal limits (biochemical response) and serum HBV-DNA maintained at <5 pg/ml (virological response) during therapy and during the entire follow-up period;

2) breakthrough indicated a continuous reappearance of serum HBV-DNA e/o increased ALT levels after an initial virological response;

3) relapse indicated serum HBV-DNA >5 pg/ml and/or increased serum ALT levels after the end of the treatment;

4) no response indicated elevated serum ALT levels and the continual presence of >5 pg/ml HBV-DNA during treatment.

All the patients were followed-up for a further 52 weeks.

The patients were examined every 4 weeks during the treatment period and every 12 weeks during follow-up. The symptoms and side effects were assessed at each control visit, as also were hepatic, pancreatic and haematological functions.

The quantitative determination of HBV-DNA was performed at weeks 12, 24, 36 and 52 of treatment and at weeks 12, 24, 36, 52 of follow-up. A second liver biopsy for histological comparison with the pre-treatment biopsy was performed at the end of follow-up in the patients who consented. Histological response was defined as decrease in Knodell-Ishak necro-inflammatory score of ≥2 points at the end of follow-up, compared with baseline value.

The primary outcome aim was defined as undetectable HBV-DNA and ALT normalisation at the end of follow-up; secondary efficacy variables included histological response.
Statistical analysis

The primary population for efficacy analysis was the intent-to-treat population (ITT) which was defined as patients with confirmed chronic anti-HBe positive hepatitis B virus infection who were randomised to treatment; the criteria for patient inclusion in the protocol population were: positivity for HBV-DNA and increased serum ALT levels at baseline, use of trial medication according to the randomisation and protocol, and non-use of prohibited medications. Therapeutic safety was analysed according to treatment received for all patients who were given at least one dose of study medication (“as treated population”).

Analysis of criteria variance was used to assess the similarity of the two groups. The Wilcoxon coupled-groups test was used to assess the response to treatment, and the Student-Newman-Keuls test was used to compare the 2 groups. A P value of <0.05 was considered significant.

RESULTS

Study population

The study was conducted between June 2000 and October 2003. A total of 67 patients were screened and 59 were randomised to treatment. Among the patients screened who were ineligible for randomisation, the majority were either HBV-DNA negative and/or did not meet the ALT inclusion criteria, thus reflecting the fluctuating levels of HBV-DNA and ALT in this patient population. The three groups of treated patients were similar in age, stage of hepatic pathology, serum ALT levels and viremia (Table 1). Treatment withdrawals due to adverse events included 3/21 patients in group A, 2/20 patients in group B and 2/18 patients in group C. The patients who were HBV-DNA positive and/or without a 50% reduction in viremia or ALT normalization at week 24 were withdrawn from the study.

ALT levels

The serum ALT values were assessed at week 12, 24, 36, at the end of treatment (week 52) and at the end of follow-up. In group A at week 12, 13/21 patients (61.9%) had a complete response and two (9.5%) a partial response (reduction ~50%); these values remained the same at week 24 and 36. At week 52, the 13 patients (61.9%) still showed ALT normalization, whereas the remaining two patients had values similar to those at baseline (p<0.05).

In group B at week 12, 14/20 patients (70%) had a complete response and three (15%) a partial response; at week 24, two of the partial responders and one total responder showed breakthrough; the remaining patient with a partial response had normal values. These results were maintained at the end of the 52-week treatment period; therefore 14/20 (70%) patients presented complete response (p<0.05).

In group C at week 12, 13/18 patients (72.2%) had a complete response and 2/18 (11.1%) showed a partial response; at week 24, the partial responder patients demonstrated breakthrough. At week 52 there were no changes in ALT serum levels; therefore 13/18 (72.2%) patients presented complete response (p<0.05).

In the follow-up period, six responders in group A, 7 in group B and 7 in group C re-

<table>
<thead>
<tr>
<th></th>
<th>Group A (21 pts)</th>
<th>Group B (20 pts)</th>
<th>Group C (18 pts)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age(years) mean</td>
<td>44</td>
<td>42</td>
<td>45</td>
</tr>
<tr>
<td>(range)</td>
<td>(27-63)</td>
<td>(23-61)</td>
<td>(26-63)</td>
</tr>
<tr>
<td>Sex (M/F)</td>
<td>(10/11)</td>
<td>(10/10)</td>
<td>(12/6)</td>
</tr>
<tr>
<td>ALT mean (U/L, n.v.&lt;40)</td>
<td>279</td>
<td>313</td>
<td>256</td>
</tr>
<tr>
<td>(range)</td>
<td>(112-357)</td>
<td>(126-389)</td>
<td>(99-357)</td>
</tr>
<tr>
<td>HBV DNA mean (pg/ml)</td>
<td>675</td>
<td>714</td>
<td>763</td>
</tr>
<tr>
<td>(range)</td>
<td>(212-975)</td>
<td>(202-1009)</td>
<td>(276-885)</td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>2</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Mild hepatitis</td>
<td>10</td>
<td>10</td>
<td>8</td>
</tr>
<tr>
<td>Moderate/severe hepatitis</td>
<td>9</td>
<td>8</td>
<td>7</td>
</tr>
</tbody>
</table>
lapsed with a return to pathologic serum ALT levels within 3 months after the end of the treatment; however, the average serum ALT levels in these patients were lower than at baseline. The overall percentage of biochemical sustained response in the three groups was 33.3% versus 35.0% versus 33.3% for groups A, B, and C, respectively, without any significant difference (p < 0.05) in the serum ALT levels between groups at the end of follow-up. P values are referred to basal ALT basal values.

**Hepatitis B virus DNA and hepatitis B surface antigen**

The average HBV-DNA values were considerably greater than 5 pg/mL in all the patients before starting therapy. In group A at week 12, 15/21 patients (71.4%) had serum virus levels <5 pg/mL; one patient had an undetectable viral load at week 24. One of the responders demonstrated a viremic breakthrough at week 24 and another at week 36. At the end of treatment, HBV-DNA was <5 pg/mL in 14/21 patients (66.7%), (p<0.05); the non-responders presented baseline HBV-DNA levels ranging from 67 to 540 pg/mL.

In group B at week 12, 14/20 patients (70%) had serum virus levels <5 pg/mL; none of the viremia level variations significant during the remaining weeks of treatment (p<0.05); the non-responders presented baseline HBV-DNA values similar to the non-responders of group A.

In group C at week 12, 14/18 patients (77.8%) had a complete response with serum virus levels <5 pg/mL. One (5.5%) patient presented a partial response (HBV-DNA ≤50% of baseline serum levels). At week 24 a complete responder patient showed a viremic breakthrough. At week 52, there were no changes in the viremia levels, therefore at the end of treatment 13/18 (72.2%) patients presented complete response. During follow-up, seven patients in group A, in group B and in group C with a viremic response experienced a relapse within 3 months after terminating treatment and the viremia returned to baseline values. After treatment withdrawal, one patient in group A demonstrated an HBV-DNA value of 5600 pg/mL at week 11 which then fell progressively and returned to the pre-treatment value by week 24; no further significant changes were observed during the following weeks of follow-up.

The percent of virological sustained response in the three groups was similar to the biochemical sustained response: 33.3% versus 35.0% versus 33.3% for groups A, B and C, respectively. None of the patients in the three groups presented HbsAg clearance.

Table 2 evidenced the rate of complete response (normal ALT and undetectable HBV DNA) in the three studied group.

**Histology**

Liver biopsy to evaluate the histological response was performed at the end of follow-up in the 20 sustained responder patients of the three groups and four, five, and four relapsed patients from groups A, B and C, respectively; the remaining patients refused to undergo a second biopsy. Assessment of the biopsy specimen was based on an eventual reduction of the HAI score of ≥2 points below the baseline histological necroinflammatory score, according to the Knodell-Ishak classification. The histological assessment was made by a single histopathologist who was unaware of the study protocol.

Five of the patients treated with LAM alone (23.8%), six (30%) treated with LAM+IFN and five (27.7%) treated with LAM+IFN in sequential therapy showed a reduction in HAI score of ≥2 points; all these subjects had previously demonstrated a sustained biochemical and virological response.

Histological activity decreased from 13 ± 0.5 at pre-treatment to 10 ± 0.9, the piecemeal necrosis (interface hepatitis) decreased from 2.5 ± 0.3 to 1.4 ± 0.3 and portal inflammation improved. In these patients, the cytoplasmic hepatitis B core antigen (HBCAg) disappeared (p <0.05). Fibrosis was improved in the same patients who

<table>
<thead>
<tr>
<th></th>
<th>Group A</th>
<th>Group B</th>
<th>Group C</th>
</tr>
</thead>
<tbody>
<tr>
<td>12 weeks</td>
<td>13/21 (61.9%)</td>
<td>14/20 (70%)</td>
<td>13/18 (72.2%)</td>
</tr>
<tr>
<td>52 weeks</td>
<td>13/21 (61.9%)</td>
<td>14/20 (70%)</td>
<td>13/18 (72.2%)</td>
</tr>
<tr>
<td>52 weeks follow-up</td>
<td>7/21 (33.3%)</td>
<td>7/20 (35%)</td>
<td>6/18 (33.3%)</td>
</tr>
</tbody>
</table>

Table 2 - Complete response (normal ALT and undetectable HBV DNA) in the three studied groups.
demonstrated a reduction of ≥2 points in the necro-inflammatory score. In the remaining patients, alterations were not significant except for the worsening of the histological findings (HAI score increase ≥2 points) in three (14.2%) group A patients, three (15%) in group B and two (11.1%) in group C. See Table 3.

**Genotype mutations**
The incidence of YMDD HBV variants was evaluated in 12 patients (4 in group A, 4 in group B and 4 in group C) who did not respond to treatment or relapsed. The 25% incidence of genotypic mutations in the YMDD locus which confers a lesser sensitivity to lamivudine: 3/12 patients (2 in group A and 1 in group B) had mutations of both codon 528 (from leucine to methionine) and codon 552 (from methionine to valine). YMDD HBV variants were not detected in any serum sample from the combination therapy-treated patients.

**Safety observations**
The safety analysis was performed in all patients who received the study medication (as-treated population). The adverse events most commonly observed in 42% of patients, were malaise, fatigue, headache, nausea, and abdominal discomfort.

There were no deaths and none of the patients presented instances of hepatic decompensation during the study. Treatment withdrawals due to serious adverse events occurred in three patients in group A, two in group B and two in group C. In one patient (group A), therapy was interrupted because of a CPK increase from normal to 2451 at week 29; thereafter, the CPK fell dramatically.

Another six patients showed hyperamylasemia with increased serum levels in pancreatic isozymes. The USG examination which was normal at baseline showed signs of focal pancreatitis; after therapy withdrawal, serum enzyme levels and results of the ultrasonographic examination were normal.

**DISCUSSION**

In this study, we evaluated the efficacy and tolerability of lamivudine alone vs α-IFN plus lamivudine in chronic anti-HBe positive HBV patients with a persistently high viral load and high ALT levels despite a previous 2 or 3 cycles of α-IFN treatment. The focus of antiviral therapy in HBV infection is the virological response to treatment as shown by changes in quantitative HBV-DNA, the biochemical response as shown by changes in ALT, and histological improvement due to the effect of viral replication suppression.

Currently, monotherapy with IFN or lamivudine has not been particularly effective for treatment of chronic hepatitis B infection; in fact, the percentage of sustained response (biochemical and virological) is achieved in only approximately 10% of these patients. Therefore, since the two drugs have different mechanisms of action, it was reasonable to ask if combination treatment with α-Interferon (immunomodulatory effect) + lamivudine (anti-viral properties) might be more effective than monotherapy in chronic anti-HBe positive HBV infection.

Previous studies have suggested that serum pre-treatment levels of viral replication as indicated by quantitative HBV-DNA values might be a strong response-predictive variable [18, 19]. Lamivudine seems to be responsible for the reduction of serum viral DNA and this is a key factor for improving the efficacy of IFN as a high viral load may be related to inefficient T-cell reactivity against HBV; while reducing the HBV DNA concentration, lamivudine may help to overcome cytotoxic T lymphocyte hypo-responsiveness and restore T-cell reactivity against HBV thus improving the efficacy of IFN [20, 21].

In this study, patients treated with combination therapy were divided in two groups, one with concomitant initiation of the two drugs and the other with lamivudine pre-treatment for 12 weeks, to evaluate which protocol is the most effective for viral load reduction. In agreement
with previous lamivudine studies both in HBeAg positive and in pre-core mutant HBV patients, we found a high percentage of patients who presented a rapid and effective reduction of HBV-DNA and corresponding normalization of ALT which was maintained throughout the treatment period [5, 12]. While the combination of lamivudine and α-interferon appeared to be associated with a higher HBeAg seroconversion and normalization of ALT when compared to lamivudine monotherapy; in our study and in agreement with others, no additional effect due to the interferon and lamivudine combination therapy was noted on the HBV-DNA clearance and ALT normalization rate [13, 14, 16, 17].

In our study HBV DNA has been determined by sandwich hybridization testing for nucleic acid (sensibility >5 pg/ml). It could determine an amplification of virological response rate. In fact, both the LAM/IFN combination and LAM monotherapy regimes appeared to effectively suppress HBV replication and provided a similar response rate (~30%) after follow-up; in addition, one-half of the responders in groups A and B relapsed after therapy discontinuation, irrespective of treatment regimen. Moreover, the 12-week pre-treatment with LAM for 12 weeks did not appear to increase the efficacy of combination therapy and the rate of sustained response in group C was similar to groups A and B.

Some recent reports have evidenced the role of combination therapy in preventing the emergence of YMDD variants in patients with chronic hepatitis B compared to LAM monotherapy, most likely due to the cumulative antiviral activity of the two drugs [13, 14, 17]. The synergistic antiviral activity provided by combination therapy could more rapidly decrease the viral load, thus reducing the possibility that YMDD mutants emerge and might be selected during LAM-treatment. Our study seems to confirm the role of combination therapy in preventing YMDD-mutants; in fact, only one patient in groups B and C, but two in the LAM treated group, developed HBV resistance due to the selection of YMDD variants.

Liver biopsy to evaluate the histological response was performed at the end of follow-up in the major part of patients (33/59, 55.9%). The histological response resulted similar in the three groups.

In conclusion, our study demonstrates that in anti-HBe positive chronic hepatitis B, a 12-month course of LAM/IFN combination therapy is as beneficial as LAM monotherapy and also that combination therapy for 40 weeks after a 12-week pre-treatment with lamivudine does not increase the rate of sustained response. Combination therapy seems more effective in preventing the emergence of YMDD variants, but this potential benefit should be further investigated in other studies.

**Key words:** lamivudine, interferon, HBV, chronic hepatitis

---

**SUMMARY**

The aim of our study was to assess the efficacy and tolerability of lamivudine alone versus lamivudine plus alpha-interferon for treatment of chronic active hepatitis B, anti-HBe positive. In all, 59 patients were randomly divided into 3 groups: A) 21 patients received lamivudine at 100 mg/daily orally for 52 weeks; B) 20 patients received lamivudine at 100 mg/die plus alpha-interferon at 6 MU subcutaneously three times weekly for 52 weeks; C) 18 patients received the same combination therapy for 40 weeks after pre-treatment with lamivudine for 12 weeks. The complete sustained response in the three groups was 33.3% vs 35.0% vs 33.3%, respectively. Our study demonstrates that in anti-HBe positive chronic hepatitis B a 12-month course of lamivudine plus α-interferon combination therapy is as beneficial as lamivudine monotherapy. Moreover, the combination therapy for 40 weeks after pre-treatment with lamivudine for 12 weeks did not increase the sustained response.
RIASSUNTO

Obiettivo: valutare l’efficacia e la tollerabilità di lamivudina verso lamivudina associata ad α-interferon per il trattamento dell’epatite cronica B, anti-HBe positiva. Pazienti e metodi: sono stati inclusi 59 pazienti. I pazienti sono stati randomizzati e divisi in tre gruppi: A) 21 pazienti trattati con lamivudina a 100 mg/die per 52 settimane; B) 20 pazienti trattati con lamivudina a 100 mg/die associata ad α-interferon al dosaggio di 6 MU sottocute tre volte la settimana per 52 settimane; C) 18 pazienti trattati con la stessa terapia combinata per 40 settimane dopo un pre-trattamento con lamivudina per 12 settimane. Risultati: la risposta completa sostenuta nei tre gruppi fu rispettivamente del 33.3%, 35% e 33.3%.

Conclusioni: il nostro studio dimostra che nei soggetti affetti da epatite cronica anti-HBe positiva un trattamento combinato di 12 mesi con lamivudina e α-interferon determina una risposta sostenuta sovrapponibile alla monoterapia con lamivudina e la stessa terapia combinata per 40 settimane dopo pre-trattamento con sola lamivudina non incrementa la percentuale di risposta sostenuta.

REFERENCES