PREDICTIVE FACTORS FOR RESPONSE TO LAMIVUDINE IN CHRONIC HEPATITIS B

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SUMMARY

Background: Lamivudine has been shown to be an efficient drug for chronic hepatitis B (CHB) treatment.

Aim: To investigate predictive factors of response, using a quantitative method with high sensitivity.

Methods: We carried out a prospective trial of lamivudine in 35 patients with CHB and evidence for viral replication, regardless to their HBeAg status. Lamivudine was given for 12 months at 300 mg daily and 150 mg thereafter. Response was considered when DNA was undetectable by PCR after 6 months of treatment. Viral replication was monitored by end-point dilution PCR. Mutation associated with resistance to lamivudine was detected by DNA sequencing in non-responder patients.

Results: Response was observed in 23/35 patients (65.7%) but only in 5/15 (33.3%) HBeAg positive patients. Only three pre-treatment variables were associated to low response: HBeAg (p = 0.006), high viral load (DNA-VHB > 3 x 10^6 copies/ml) (p = 0.004) and liver HBcAg (p = 0.0028). YMDD mutations were detected in 7/11 non-responder patients.

Conclusions: HBeAg positive patients with high viral load show a high risk for developing drug resistance. On the other hand, HBeAg negative patients show a good response to lamivudine even with high viremia.

KEYWORDS: Hepatitis B; HBV; Lamivudine; HBV-DNA; PCR; bDNA

INTRODUCTION

Chronic hepatitis caused by hepatitis B virus (HBV) may lead to cirrhosis, liver failure and hepatocellular carcinoma. The current treatment with interferon-α (IFN-α) remains unsatisfactory, as it is effective in fewer than 40 percent of patients4,6, and previous treatment with corticosteroid is still in use to improve results6,13.

Lamivudine (2’, 3’-dideoxy-3’-tiacytidine) is a potent inhibitor of viral DNA polymerase and is considered one of the most promising nucleoside analog. Following entering the cells, lamivudine is phosphorylated to its active metabolite, lamivudine-5’-triphosphate, by the 2’-deoxycytidine kinase and other cellular kinase enzymes35. It, then, interferes with the reverse-transcriptase activity of the HBV polymerase, terminating the nascent viral DNA chain and inhibiting DNA synthesis37.

According to a preliminary study carried out by DIENSTAG et al., daily doses of 100 and 300 mg of lamivudine reduced HBV-DNA to undetectable levels in all patients during a six month therapy; but sustained undetectable levels of HBV-DNA and hepatitis-B e antigen (HBeAg) occurred in only 5-12% of the treated patients6. In an extended study for up to 18 months, with 23 patients who had remained HBeAg positive after six months of therapy, the same research group observed that HBV-DNA suppression was maintained during therapy in 20 (87%) of these patients. Whereas, HBeAg became negative in 39% of the patients7.

A recent report showed that HBV-DNA levels in serum may decrease below the detection threshold of the Polymerase Chain Reaction (PCR) assay in 46% of 51 patients, after a 24-week course of lamivudine16. A trend towards a deeper suppression of viral replication with a daily dose of 300 mg was also observed12. Such efficacy of lamivudine to impair HBV replication was also shown in HIV-infected9 and in transplanted patients13, 28, 29.

Daily doses of lamivudine in patients with chronic hepatitis B (CHB) has varied widely, ranging from 5 to 600 mg. Doses above 5 mg reproducibly decreased HBV-DNA levels in serum9, but doses of 100 mg and 300 mg can reduce viremia to an undetectable level9. Daily doses...
of 300 mg may lead to an earlier and more profound suppression of HBV DNA.

Viral resistance to lamivudine is associated with mutations which lead to amino acid substitutions in the highly conserved Tyr-Met-Asp-Asp (YMDD) motif, in the active site of the polymerase. The appearance of such mutation (Methionine to Isoleucine or Valine) may explain the viral “breakthrough” during lamivudine therapy.

Despite the reported efficacy of lamivudine in patients not previously treated and in those who had not responded to interferon therapy, additional data are needed to evaluate whether there is a difference in response to lamivudine in these two groups of patients.

The aim of this study was to assess the efficacy and safety of lamivudine in two groups of Brazilian patients with different degrees of CHB: one who had not been previously treated with any drug (12 patients), and another after failure of IFN-α treatment (23 patients).

The present study revealed an as yet not described correlation between viral load and response to lamivudine.

**PATIENTS AND METHODS**

**Patients**

We carried out an open and prospective trial of lamivudine in 35 consecutive patients with CHB with evidence of viral replication. Eligible patients included 30 men and 5 women, ranging from 9 to 72 years old. All had hepatitis B surface antigen (HBsAg) in serum for at least six months, detectable HBV-DNA in serum (34 by PCR and one by bDNA) and alanine aminotransferase (ALT) levels ranging from normal levels to 10 times the upper normal limit (UNL).

The inclusion criteria were broad, allowing the enrollment of patients with low transaminase levels as well as patients with low degree of hepatitis, on one side, and patients with compensated (Child-Pugh A) and decompensated (Child-Pugh B) cirrhosis on the other side.

Patients were excluded if they were also infected with hepatitis C virus, hepatitis D virus or human immunodeficiency virus (HIV), had other serious medical illness, another type of liver disease or an advanced decompensated liver disease (Child-Pugh C), or were pregnant or lactating.

Patients consecutively attended from March 1996 to July 1997 were considered for treatment with lamivudine on a daily dose of 300 mg for 12 months and 150 mg thereafter. Only 4 patients with decompensated liver cirrhosis (Child-Pugh B) received 150 mg from the beginning.

Thirty five patients have been enrolled, being 30 under lamivudine treatment and most of them for longer than 6 months (long term treatment). Lamivudine was withdrawn from five patients who presented YMDD mutation after nine months of treatment (4 with YVDD and one with YIDD mutations).

Patients were divided in two groups: Group I included 12 patients who had not been previously treated (“naive”) and, Group II, 23 patients who had been previously treated with interferon-α (IFN-α). Resistance to IFN-α was considered as partial in case of HBsAg clearance and persistence of HBV-DNA detected by PCR (15 patients). Complete resistance was defined as persistence of HBsAg and HBV-DNA (8 patients). None of these patients received immunosuppressive or antiviral therapy at least 6 months before lamivudine therapy.

**Biopsies**

Liver biopsy was performed using a Tru-Cut™ needle in 25 patients, but not in the remaining 10, due to coagulation disturbances or ascitis. In the latter cases, a clinical and ultrasonographic diagnosis of cirrhosis was performed. Liver histology was blindly evaluated by two specialized pathologists.

**Immunohistochemistry**

HBsAg and Hepatitis B core antigen (HBcAg) detection in liver tissue was carried out using monospecific polyclonal antibodies in the high-sensitive streptavidin-biotin-peroxidase system (LSAB, Dako, USA), after blockage of endogenous biotin and peroxidase.

**Serology**

Detection of HBsAg, HBeAg and antibodies to hepatitis B e antigen (anti-HBe) in serum samples were carried out by ELISA, using commercially available kits (Abbott Laboratories, North Chicago, IL, USA).

**Detection of HBV-DNA by Polymerase Chain Reaction (PCR)**

HBV-DNA was detected by a nested PCR as previously described. Serum samples (10 µl) were denatured with 2.5 µl of NaOH 0.5 M and incubated at 37 °C for one hour. Samples were neutralized with 2.5 µl of HCl 0.5 M. For the first round, 1 µM of each primer (1763 (5’TGT GGG CAT GGA CAT TGA CCC GTA TAA 3’) and 2032R (5’CTG ACT AAT TCC CTG GAT TGG TCT 3’)) were added to the mixture. Amplification was achieved in a thermocycler model 480 (Perkin Elmer, USA), in 25 cycles of 94 °C for 1 minute (min.), 42 °C for 1 min. and 72 °C for 2 min., followed by an extension step at 72 °C for 5 min. In the second round, 1:10 of the first round product was amplified by primers 1778 (5’CAT TGA CCC GTA TAA AGA ATT 3’) and 2017 (5’CTG GAT GCT GGG TCT TCC AAA 3’), in the same conditions as above. Amplified products from the second round were electrophoresed in a 2% agarose gel, stained with Ethidium Bromide and visualized under ultra-violet light.

To avoid false-positive results, strict procedures proposed for nucleic acid amplification techniques were followed.

**Detection of mutations associated with resistance to Lamivudine**

HBV-DNA positive samples were also amplified with primers
corresponding to the HBV polymerase gene, targeted to the region of mutations associated with lamivudine resistance. Serum samples were denatured and neutralized as described above and submitted to nested PCR. The outer primers 5’TGC RYY TGT A TT CCC A TC CCA TC 3’ and 3’TGT TTA GGG TTT AAA TG 3’ were used in the first round, while the inner primers L840 5’ACC CCA TCT TTT TGT TTT GTT AAG 3’ and L372 5’TGC CTG GAT GTG TCT GCG GCG TTT T A T 3’ were used in the second round. Amplification for the first and second round was carried out at 94 °C for 1 min., followed by 35 cycles of 94 °C for 30s, 42 °C for 30s and 72 °C for 40s and a final extension step at 72 °C for 7 min in a PTC-100 thermocycler (MJ Research, Watertown, MA, USA).1, 2

Sequencing

PCR products were submitted to cycle sequencing reactions, using the second round primers described above and the ABI Prism® BigDye® Terminator Cycle Sequencing Ready Reaction Kit with AmpliTaq® DNA Polymerase, FS (PE Biosystems, Foster City, CA, USA). Nucleotide sequences from both strands were determined in an Automated DNA sequencer model ABI 310 FS (PE Biosystems, Foster City, CA, USA). Sequences were compared with those previously described in the literature using the Lasergene program (DNAsstar, Madison, WI, USA).

Quantification of HBV DNA

HBV viremia was followed in sequential samples from the patients using an in-house end-point dilution PCR, or simply EPD method.

Samples were initially diluted in water to a 10-fold series till 10⁶. Each dilution was submitted to the same PCR protocol described above for detection of HBV-DNA. As the sensitivity of the method was reported to be around 3 copies per 10 µl or 300 copies/ml, quantitative results were estimated by multiplying the least positive dilution by 3 x 10² copies/ml.

Assessment of response

Patients were considered as responders if HBV-DNA was undetectable by PCR at the sixth month of treatment.

Other endpoints such as HBeAg clearance, HBeAg to anti-HBe seroconversion and disappearance of HBsAg were also investigated. HBeAg clearance was defined as the absence of HBeAg in two consecutive samples.

Statistical analysis

Univariate analysis for non-responder and responder patients were performed according to the Chi-square (χ²) and Fisher’s Exact Test, taking into consideration the following variables: sex, previous treatment with IFN-α, viremia, presence of serum HBsAg, HBeAg in situ, and liver cirrhosis. Age and pre-treatment ALT levels were analysed using the Student’s t-test. Pre-treatment ALT levels were also analysed by the non-parametric Mann-Whitney-Wilcoxon test. The logistic regression technique was performed to estimate the probability of non-response based on variables that demonstrated statistical significance in the univariate analyses. The stepwise method was used to select the variables more associated with the probability of non-response.

The level of significance was 5% (α = 0.05); and the SAS software (Statistical Analysis System, SAS Institute, NC, USA) was used.

All patients gave informed consent for the study, which was approved by the Local Ethics Committee.

RESULTS

Therapy efficacy

Lamivudine therapy induced a rapid decrease in serum HBV-DNA concentration in 27 out of 29 patients (93.1%) whose quantification was performed after 12 weeks of treatment: 14 (51.9%) had undetectable HBV-DNA, and 13 (48.1%) showed a decrease in viremia. The other 2 patients presented no change in the HBV-DNA concentration (Table 1 and Table 2). At the sixth month of treatment, chosen as the time point to define response in this study, HBV-DNA was undetectable in the serum.

Table 1

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age (years)</th>
<th>Group</th>
<th>PCR (log genomes/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Pre</td>
</tr>
<tr>
<td>1</td>
<td>M</td>
<td>17</td>
<td>I</td>
<td>3</td>
</tr>
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<td>3</td>
<td>M</td>
<td>29</td>
<td>I</td>
<td>4</td>
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<td>5</td>
<td>M</td>
<td>72</td>
<td>I</td>
<td>10</td>
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<td>6</td>
<td>M</td>
<td>62</td>
<td>I</td>
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<td>8</td>
<td>F</td>
<td>9</td>
<td>I</td>
<td>8</td>
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<td>9</td>
<td>M</td>
<td>59</td>
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<td>9</td>
</tr>
<tr>
<td>10</td>
<td>M</td>
<td>47</td>
<td>I</td>
<td>4</td>
</tr>
<tr>
<td>12</td>
<td>M</td>
<td>67</td>
<td>I</td>
<td>6</td>
</tr>
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<td>3</td>
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<td>4</td>
</tr>
<tr>
<td>35</td>
<td>M</td>
<td>45</td>
<td>II</td>
<td>6</td>
</tr>
</tbody>
</table>

Sex (M = male, F = female); Age (years); Group (I = no previous treatment; II = previous treatment with Interferon-α); bDNA = bDNA; ND = not done; NEG = negative.
For this reason, the variable log of number of genomes ($G$), as determined by EPD-PCR, was selected and the model was written as follows, where $p$ is the probability of non-response to lamivudine.

$$p = \frac{\exp(-3.507 + 0.387G)}{1 + \exp(-3.507 + 0.387G)}$$

In this model, the variable $G$ assumes continuous values from 3 to 12. The estimated odds-ratio (1.473; 95% confidence interval, 1.109 to 1.958) showed that the chance of non-response increases 1.5 times for each increase of one log in the viral load, as shown in Figure 1.

All but one non-responder patients at the sixth month persisted as PCR-positive at the twelfth month, regardless of their viremia level by PCR. On the other hand, all but one responder patients were PCR negative at the twelfth month.

### Serum HBV markers

At the sixth month of therapy, HBeAg became negative in only 3 (20%) out of 15 patients. Among the remaining 12 HBeAg positive, 5 (42%) became negative after one year of therapy. HBeAg negativation was followed by anti-HBe seroconversion in all responder patients; in one patient, HBeAg disappeared only after 21 months of therapy. Interestingly, despite the detection of HBV-DNA and YVDD mutation, the HBeAg antigen became negative in one non-responder. Moreover, HBsAg clearance was observed in only one responder patient, after 20 months of therapy.

### Table 2

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Group</th>
<th>Pre</th>
<th>12 weeks</th>
<th>24 weeks</th>
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<tbody>
<tr>
<td>2</td>
<td>F</td>
<td>22</td>
<td>I</td>
<td>10</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>42</td>
<td>I</td>
<td>10</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>M</td>
<td>51</td>
<td>I</td>
<td>12</td>
<td>NEG</td>
<td>4</td>
</tr>
<tr>
<td>11</td>
<td>M</td>
<td>11</td>
<td>I</td>
<td>10</td>
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<td>4</td>
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<td>13</td>
<td>M</td>
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<td>6</td>
</tr>
<tr>
<td>16</td>
<td>M</td>
<td>30</td>
<td>II</td>
<td>8</td>
<td>NEG</td>
<td>4</td>
</tr>
<tr>
<td>17</td>
<td>M</td>
<td>65</td>
<td>II</td>
<td>12</td>
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<td>M</td>
<td>44</td>
<td>II</td>
<td>10</td>
<td>ND</td>
<td>3</td>
</tr>
<tr>
<td>22</td>
<td>F</td>
<td>23</td>
<td>II</td>
<td>3</td>
<td>ND</td>
<td>4</td>
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<td>29</td>
<td>M</td>
<td>60</td>
<td>II</td>
<td>10</td>
<td>6</td>
<td>4</td>
</tr>
</tbody>
</table>

Sex (M = male, F = female); Age (years); Group (I = no previous treatment; II = previous treatment with Interferon-$\alpha$); ND = not done; NEG = negative.

### Levels of viral load at pre-treatment were correlated with response to lamivudine in two ways: when comparing patients with high ($>3 \times 10^6$ genomes/ml) and low viremia ($\leq 3 \times 10^6$ genomes/ml) ($p = 0.004$), and when analyzing the log of number of such values using a non-parametric test ($p = 0.006$).

The stepwise method selected two variables associated with response to lamivudine: high or low viremia (genomes/ml) and level of viremia (log genomes/ml). With the first variable, the probability of non-response to lamivudine is 11.4 times higher in patients with high viremia ($>3 \times 10^6$ genomes/ml), with a very wide 95% confidence interval (1.938 to 66.355). For this reason, the variable log of number of genomes ($G$), as determined by EPD-PCR, was selected and the model was written as follows, where $p$ is the probability of non-response to lamivudine.
Serum alanine aminotransferase (ALT) activity:

Serum ALT activity was determined in 33 patients at the sixth month. Of 18 patients with increased ALT level at baseline, 12 became normal (including three non-responders). Whereas of 15 patients with normal ALT at baseline, only one non-responder showed an increased ALT level after 6 months of treatment. Therefore, lamivudine was equally effective at suppressing viral load among patients with normal and elevated ALT levels (Table 3).

The seven patients with lamivudine resistance and YMDD mutations (see below) showed a slight elevation of the ALT level (0.5 to 3.0 times the upper normal limit) with abnormal values in four of them. No biochemical flare was observed, except in one patient during an one-month association of famciclovir.

Table 3

<table>
<thead>
<tr>
<th>Variable</th>
<th>Responder (%)</th>
<th>Non-responder (%)</th>
<th>Total</th>
<th>p</th>
<th>Test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>20 : 3</td>
<td>10 : 2</td>
<td>35</td>
<td>1.00</td>
<td>Fisher</td>
</tr>
<tr>
<td>Age (mean; SD)</td>
<td>41.4 ; 17.5</td>
<td>37.3 ; 16.8</td>
<td>35</td>
<td>0.51</td>
<td>Student’s</td>
</tr>
<tr>
<td>HBeAg positive</td>
<td>6 (40)</td>
<td>9 (60)</td>
<td>15</td>
<td>0.006</td>
<td>χ²</td>
</tr>
<tr>
<td>HBeAg negative</td>
<td>17 (85)</td>
<td>3 (15)</td>
<td>20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-cirrhosis</td>
<td>15 (65)</td>
<td>8 (35)</td>
<td>23</td>
<td>1.00</td>
<td>Fisher</td>
</tr>
<tr>
<td>Cirrhosis</td>
<td>8 (67)</td>
<td>4 (33)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previous IFN-α: Yes</td>
<td>15 (65)</td>
<td>8 (35)</td>
<td>23</td>
<td>1.00</td>
<td>Fisher</td>
</tr>
<tr>
<td></td>
<td>8 (76)</td>
<td>4 (33)</td>
<td>12</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pre-treatment ALT (x UNL) (mean; SD)</td>
<td>2.66 ; 2.95</td>
<td>1.33 ; 0.86</td>
<td>35</td>
<td>0.054</td>
<td>Student’s</td>
</tr>
<tr>
<td>Pre-treatment ALT (x UNL (&quot;)) (median)</td>
<td>1.26</td>
<td>0.97</td>
<td>35</td>
<td>0.37</td>
<td>Mann-Whitney-Wilcoxon</td>
</tr>
<tr>
<td>Viral load (median of log genomes/ml)</td>
<td>4</td>
<td>10</td>
<td>34 (&quot;)</td>
<td>0.006</td>
<td>Mann-Whitney-Wilcoxon</td>
</tr>
<tr>
<td>Viremia(genomes/ml):</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>High (&gt;3x10⁶)</td>
<td>7 (41)</td>
<td>10 (59)</td>
<td>17</td>
<td>0.004</td>
<td>χ²</td>
</tr>
<tr>
<td>Low (≤3x10⁶)</td>
<td>15 (88)</td>
<td>2 (12)</td>
<td>17</td>
<td></td>
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<td>HBeAg in situ:</td>
<td></td>
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<tr>
<td>Positive</td>
<td>4 (40)</td>
<td>6 (60)</td>
<td>10</td>
<td>0.028</td>
<td>Fisher</td>
</tr>
<tr>
<td>Negative</td>
<td>13 (87)</td>
<td>2 (13)</td>
<td>15</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

(" One patient was excluded because his pre-treatment viral load was determined only by bDNA. SD = Standard Deviation; UNL = upper normal limit.

Previous treatment with Interferon-α

As shown in Table 3, no difference was found between Group I (naive of treatment) and Group II [resistant to Interferon-α (IFN-α)]. However, considering the pattern of previous response to IFN-α, we observed a trend (Fisher’s Exact Test: p = 0.058) to a better response to lamivudine in patients who had shown a partial response (12 out of 15 patients) as compared to those who did not respond (3 out of 8 patients) to IFN-α, respectively.

As for HBV-DNA levels, a baseline high viremia was detected in 8 out of 12 patients naive of treatment and in 9 out of 23 interferon-resistant patients, with a response to lamivudine of 50% and 33%, respectively. On the other hand, a baseline low viremia was observed in 4 out of 12
naive patients and in 14 out of 23 Interferon - resistant patients, with a response frequency of 100% and 86%, respectively. No statistical significant difference was found in both cases.

Resistance to Lamivudine

Of the 12 non-responders to lamivudine, samples of 11 were sequenced for the YMDD motif of the C region, and 7 (64%) of them showed mutations associated with lamivudine resistance. Six (55%) patients showed a YVDD mutation and one (9%) of them a YIDD mutation. The other 4 (36%) patients presented the wild type virus, and in the 12th patient, sequencing was not possible as no DNA amplification of this region was obtained after several PCR attempts.

At the time samples were collected for sequencing the YMDD motif, 8 out of the 11 patients were under lamivudine treatment (period of 6 to 24 months, average 12.5 months). In the other 3 patients, treatment had been withdrawn at 2 (one patient) and 8 months (2 patients) before the mutation analysis, and all showed the wild type YMDD motif.

All 7 patients with resistance mutation were HBeAg positive at baseline. A detailed analysis of YMDD mutations during and after withdrawal of lamivudine will be described elsewhere.

DISCUSSION

The recent introduction of the drug lamivudine added a new expectative to the treatment of Chronic Hepatitis B (CHB). In the present study, in agreement with previous results using Abbott Genostics liquid hybridization assay[6,26], a gradual or a rapid decrease in HBV-DNA levels was observed. This pattern was detected by the three different quantitative methods: end-point dilution (EPD), Amplicor Monitor and branched PCR assays will be useful for evaluating new drugs and therapy regimens[31] and that a negative PCR at the sixth month is an useful criterion for determining response to lamivudine.

Our results also showed that non-response to lamivudine is mainly observed in serum of patients with high viremia and, most of them, positive for HBeAg. This correlation was so significant that a proportion between probability of non-response and increase in the logarithm of genome copies per ml could be established (Figure 1). MUTIMER et al.24 showed that high titers of hepatitis B virus in serum of pre-treated patients predicted failure of lamivudine prophylaxis and graft re-infection after liver transplantation.

Mutations in the YMDD motif were observed only in non-responder patients with high viremia and positive HBeAg. If we take into consideration only the first 12 months of treatment, 33% of our patients (5/15) presented such mutation - which is comparable to the frequency obtained by HONKOOP et al. (39%, in 14 patients)20 - both within the same therapy period. Furthermore, if we consider the full length of our treatment (24 months), the frequency of mutation carriers was even higher: 47% (7 out of 15 patients).

In a later paper, HONKOOP et al. calculated an actuarial cumulative incidence of YMDD mutation carriers of 32% and 53% after 52 and 78 weeks, respectively, of lamivudine therapy in 15 patients treated for more than 12 months[4].

According to LAU et al., lamivudine resistance tends to appear specially in patients whose serum levels of HBV-DNA persist above 1,000 Eq/ml[22].

The response frequency found in HBeAg-negative patients was 85%; higher than that reported by TASSOUPOULOS et al. patients with the same serological profile (63%), probably because we included patients with low viremia, not detected by the bDNA method employed by these workers[31]. Interestingly, 27% of patients in the Tassoupoulos’ study showed YMDD variant at week 52. This mutation was not observed by us in HBeAg negative patients.

Supporting data for the hypothesis that persistent low degree of viremia (detected only by PCR quantitative methods) is clinically relevant were shown by the evolution of our non-responder patients at the sixth month. All but one of the 12 non-responder patients persisted with positive PCR and a viral load from 10^3 to 10^7 genomes/ml and out of these 11, seven patients showed a progressive increase of viremia and a resistance mutation in the YMDD motif. Conversely, all but one of the 23 responder patients (PCR negative at the sixth month) persisted as such after 12 months of therapy. These results suggest that highly sensitive PCR assays will be useful for evaluating new drugs and therapy regimens31 and that a negative PCR at the sixth month is an useful criterion for determining response to lamivudine.

It is worth mentioning that one patient became HBeAg negative, despite the detection of a YVDD mutation to lamivudine resistance. This fact was also observed by GARRETT et al.2.

Another important point is that the response to lamivudine did not depend on the presence or absence of liver cirrhosis. Furthermore, the tolerance to lamivudine was equally good in both situations, even in patients with ascitis (Child Pugh B).
Summing up, two important conclusions arose from our observations: first, patients positive for HBeAg and with a high viral load are less prone to respond to lamivudine as monotherapy due to the high risk of drug resistance and selection of mutation in the YMDD motif; second, patients negative for HBeAg generally showed good response to lamivudine, even in case of liver cirrhosis or of high viremia. A longer follow-up for these and other patients with YMDD mutation will be described elsewhere.

RESUMO

Fatores preditivos para resposta da lamivudine na hepatite crônica B

Introdução: A Lamivudina tem-se mostrado útil no tratamento da hepatite crônica pelo vírus B (HC-VHB).

Objetivo: Investigar os fatores preditivos da resposta à Lamivudina na HC-VHB.

Material e Métodos: Estudo prospectivo com Lamivudina em 35 pacientes com HC-VHB e evidência de multiplicação viral, independentemente do resultado do AgHBe. Administrou-se a Lamivudina na dose diária de 300 mg por 12 meses, seguida de 150 mg diários. Critério de resposta: DNA-VHB negativo (por técnica de PCR) aos 6 meses de tratamento. Nos pacientes não respondedores, pesquisaram-se mutações associadas com resistência à Lamivudina, através do sequenciamento do DNA viral.

Resultados: Observou-se resposta em 23/35 pacientes (65,7%). Dos 15 pacientes com AgHBe positivo antes do tratamento, apenas 5 (33,3%) responderam. As variáveis prévias ao tratamento que puderam prever uma má resposta foram: AgHBe positivo (p = 0,006), carga viral elevada (> 3 x 10^4 genomas/ml) (p = 0,004) e AgHBc no tecido positivo (p = 0,0028). Mutações na região YMDD foram detectadas em 7/11 pacientes não respondedores.

Conclusões: Pacientes com AgHBe positivo e com alta carga viral apresentam um alto risco de desenvolver resistência à Lamivudina. Por outro lado, pacientes com AgHBe negativo, mesmo com alta carga viral, mostraram uma boa resposta à Lamivudina.

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