

# Initial High Dose of Lamivudine Delays the Appearance of Viral Resistance in Chronic Hepatitis B Patients

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## Abstract

**Background:** The lamivudine dosage used for treatment of patients with hepatitis B virus (HBV) chronic liver disease is one-third of the dose used in patients infected with human immunodeficiency virus. Moreover, lamivudine therapy is hampered by the early and high rate of drug-resistance. **Aim:** To assess the effect of an initial high dose of lamivudine on the rate and temporal incidence of the development of resistance to treatment. **Methods:** We retrospectively studied 62 patients (49 males; median age 54 years) with chronic HBV-related liver disease who were treated with lamivudine and who had at least 1-year on-treatment follow-up. Patients were subdivided according to the lamivudine dosage: 25 patients were treated with lamivudine 300mg qd for two weeks, then shifted to 100mg qd (high-dose group) and 37 patients were treated with the standard dose of 100mg qd (standard-dose group). **Results:** Median treatment duration was 45 months. As far as baseline HBV-DNA, HBeAg status, stage of disease, and previous interferon treatment are concerned there were no differences between groups. Viral resistance was detected in 43 patients (69%) after a median of 27 months (range: 6-72) with no significant difference between groups (high-dose, 60% versus standard-dose, 76%). Appearance of viral resistance was significantly delayed in the high-dose group ( $p=0.0274$ ). **Conclusions:** This study has shown that an initial high dose of lamivudine is able to delay the appearance of viral resistance in patients with chronic HBV infection, thus suggesting that the genetic barrier of lamivudine could be dose-dependent.

## Key words

Lamivudine – chronic hepatitis B – resistance.

## Introduction

Chronic hepatitis B is a heterogeneous disease that affects more than 300 million people worldwide [1]. Patients persistently infected with hepatitis B virus (HBV) may present with markedly different levels of HBV replication, and often alternate periods of absence of liver inflammation and severe hepatitis. Control and eradication of HBV infection are considered one of the major public health challenges of the 21st century [2, 3].

Lamivudine is a deoxycytidine analogue that acts as a chain terminator during reverse transcription of the pregenome and can result in a potent suppression of viral load. It was the first nucleoside analogue to be used successfully and licensed for the treatment of HBV infection [4]. The therapeutic use of nucleoside analogues to inhibit HBV DNA replication led to the selection of resistant mutants. Resistance to lamivudine is associated with point mutations that result in the substitution of the methionine residue in the YMDD motif by either valine or isoleucine (rtM204V/I) [5, 6].

The rate of selection of lamivudine-resistance viral strains increases progressively during treatment and prolonged monotherapy with lamivudine results in the emergence of resistant virus in 40% of patients after 2 years of therapy and 65% after 5 years [7]. Thus, antiviral drug resistance poses a major problem for the management of patients with chronic hepatitis B. Theoretically, resistance may be prevented if a sufficiently potent antiviral drug, or a combination of antiviral agents, is used so as to eliminate viral replication with the highest efficacy, and thereby prevent the ongoing selection of HBV quasispecies [8]. Mutations appear in a stochastic fashion and are selected in a negative way by the presence of the drug. Treatment of HBV with antiviral agents that fail to suppress viral replication completely inevitably leads to the selection of antiviral resistance mutations [9]. Mutations can arise only if the virus replicates, hence the importance of the efficacy and efficiency of the drug (which are also dose dependent) for suppressing the viral replication as well and as fast as possible. In comparison with human immunodeficiency virus

Received: 01.12.2010 Accepted: 11.01.2011

J Gastrointest Liver Dis

March 2011 Vol. 20 No 1, 47-50

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(HIV) infection, lamivudine has been used to a third of its original dosage, and a stable plasmatic level is fundamental to assure an intracellular concentration of the drug, which will be then transformed into the active form in an amount that reflects the plasma values.

The aim of this retrospective study was to evaluate the effect of an initial high dose of lamivudine - 300mg daily for two weeks - on the rate and timing of the development of lamivudine resistance during antiviral treatment.

## Patients and methods

This retrospective study included 62 patients with chronic HBV-related liver disease. Patients with other concomitant causes for chronic liver disease (hepatitis C virus infection, alcohol abuse, autoimmunity) or with HIV infection were excluded from this study. Patients were subdivided in two groups and treated as follows: 25 patients were treated with lamivudine 300 mg qd for two weeks, then shifted to 100 mg qd (high dose group) and 37 patients were treated with the standard dose of 100 mg qd (standard dose group). The decision to treat patients with an initial high dose or standard dose was left to the discretion of the physician in charge of the patient. All patients had at least 1-year on-treatment follow-up.

At baseline we collected data on demographic characteristics, risk factors for HBV acquisition, time of infection; virological profile [presence of HBV e-antigen (HBeAg) and anti-HBe antibodies; qualitative and/or quantitative HBV DNA results]; stage of liver disease, defined according to the clinical, biochemical and histological findings; previous treatment for HBV (type, duration, and outcome). Biochemical activity of disease was defined as the presence of altered aminotransferases according to the upper limit of normal in the local laboratory (i.e., 40 IU/L). Patients with biochemical activity of disease were further characterised as patients with persistent activity and patients with aminotransferases flares according to commonly accepted criteria [10].

Thereafter, clinical and biochemical data were collected at two weeks, at the first month and then monthly during treatment. Virological evaluation (qualitative serum HBV-DNA) was performed at the first and third month of treatment and every three months thereafter or when clinically indicated (i.e., in case of aminotransferase elevation). In the case of viral breakthrough, lamivudine-resistance test (INNO-LiPA HBV DR v2, Innogenetics NV, Gent, Belgium) was performed.

Serology for HBV infection was carried out by means of the standardized ELISA test. Quantitative HBV-DNA was assessed by Versant 3.0 bDNA (Bayer Corp., Tarrytown, NJ) with an upper limit of  $1.8 \times 10^7$  IU/ml. Qualitative HBV-DNA was assessed by PCR assay (COBAS Amplicor HBV Monitor test, Roche Diagnostics SpA, Milan, Italy; lower limit of detection: 60 IU/ml).

Data are reported as median and range or absolute value and percentage. Statistical analysis was carried out with

Fisher's exact test and the non-parametric Mann-Whitney U-test. Differences in the rate of appearance of lamivudine resistance during time in the two study groups were assessed by means of the Kaplan-Meier analysis. For all analyses a p value <0.05 was considered statistically significant. Data were analysed using MedCalc software version 9.2.1.0 (MedCalc Software bvba, Mariakerke, Belgium).

## Results

Table I shows the main demographic and clinical characteristics of the 62 study patients. The study population was made-up prevalently of male patients (49 patients, 79%) with community-acquired infection (57 patients, 92%) in adulthood (43 patients, 69%). Expectedly in our geographic area, the majority of patients were infected with e-minus HBV strands (49 patients, 79%). Median lamivudine treatment duration was 45 months (range 14-96 months). The main demographic, clinical, and virological characteristics of the study patients, subdivided according to the e-antigen status are shown in Table II. There were no significant differences in the main variables in the two groups of patients, though e-positive patients tended to have higher HBV DNA serum levels.

**Table I.** Main demographic and clinical characteristics of the 62 patients who made up the study population.

Parameter	Unit	Value
Gender	male	49 (79)
Age	years	54 (20-84)
Route of infection		
community acquired		57 (92)
perinatal transmission		2 (3)
blood products transfusion		3(5)
Anti-HBe antibodies	positivity	49 (79)
Timing of infection		
unknown		14 (23)
perinatal		2 (3)
adolescence		3 (5)
adulthood		43(69)
Lamivudine treatment duration	months	45 (14-96)

Data are shown as median and range (parenthesis) or as absolute value and percentage (parenthesis)

Patients characteristics were assessed on the basis of the initial dose of lamivudine received (high versus standard dose). Table III shows that as far as baseline virological (HBV DNA levels, HBeAg status) and clinical (stage of disease, previous interferon treatment) parameters were concerned we observed no statistically significant difference between groups, while patients who received an initial high dose were older than patients who received a standard dose ( $p=0.010$ ) and had received a longer follow-up ( $p=0.001$ ).

**Table II.** Demographic and clinical characteristics of the patients subdivided according to e-antigen status.

		HBe-antigen positive (n=13)	HBe-antigen negative (n=49)
Gender	male	11 (85)	38 (76)
Age	years	49 (20-83)	56 (27-84)
Follow-up	months	34.5 (17.2-95.8)	54.3 (13.6-87.1)
HBV DNA	IU/mL	1.2x10 <sup>7</sup> (2770-1.8x10 <sup>7</sup> )	4x10 <sup>5</sup> (614-1.8x10 <sup>7</sup> )
Biochemical activity			
	inactive	1 (8)	1 (2)
	flares	1 (8)	8 (16)
	persistent	11 (84)	40 (82)
Previous IFN treatment	yes	5 (38)	21 (43)

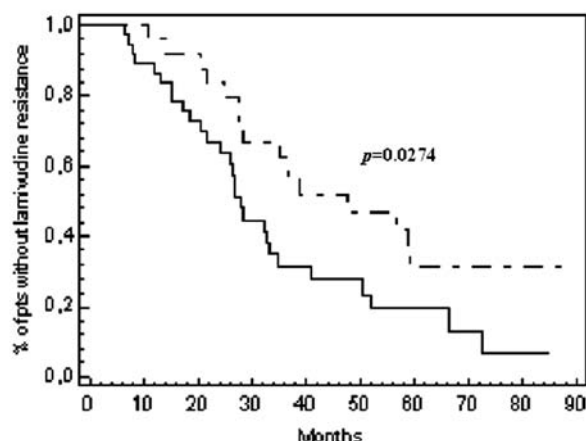
Data are shown as median and range or as absolute value and percentage; IFN, interferon; HBV, hepatitis B virus.

**Table III.** Baseline demographic and clinical characteristics of the patients subdivided according to the initial dose of lamivudine received.

		High dose (n=25)	Standard dose (n=37)	p
Gender	male	22 (88)	27 (73)	0.210
Age	years	61 (43-80)	51 (20-84)	0.010
Follow-up	months	72 (20-87)	34 (14-96)	0.001
HBV-DNA	IU/mL	1.38x10 <sup>6</sup> (2.5x10 <sup>4</sup> - 1.8x10 <sup>7</sup> )	1.4x10 <sup>5</sup> (614 - 1.8x10 <sup>7</sup> )	0.084
HBeAg positive		2 (8)	11 (30)	0.057
Biochemical activity				
	inactive	1 (4)	1 (3)	
	flares	1 (4)	8 (22)	
	persistent	23 (92)	28 (75)	0.153
Previous IFN treatment	yes	10 (40)	16 (43)	1.0

Data are shown as median and range or as absolute value and percentage; IFN, interferon; HBV, hepatitis B virus.

During treatment, genotypic viral resistance was detected in 43 patients (69%) after a median time of 27 months (range 6-72 months) with no statistically significant difference between the high (60%) and standard lamivudine dose (76%). The remaining patients were continuous responders to the treatment for a median time of 37 months (range 18-87 months). Longitudinal assessment of appearance of genotypic resistance showed that in patients treated with initial high dose lamivudine, the appearance of viral resistance was significantly delayed as compared to patients treated with standard dose lamivudine (Fig. 1), with a median time to appearance of genotypic resistance of 48 months in

**Fig 1.** The Kaplan-Meier curve analysis shows that in patients treated with an initial high lamivudine dose (dotted line) the appearance of viral resistance was significantly delayed as compared to patients treated with standard dose (solid line).

patients treated with an initial high lamivudine dose and 28 months in patients treated with a standard lamivudine dose ( $p=0.0274$ ). After the appearance of lamivudine-resistant HBV patients were treated with adefovir dipivoxil.

## Discussion

Despite the enormous efforts and the new available antiviral drugs, we are far from reaching a complete and world-wide affordable control of HBV related liver disease. Besides pegylated interferon, current treatment options include three nucleoside analogues (lamivudine, telbivudine, and entecavir) and two nucleotide analogues (adefovir dipivoxil and tenofovir disoproxil fumarate) [11]. The price of these treatments is very high and the majority of the world population affected by HBV infection cannot afford it.

The first and more affordable drug, lamivudine, has proved to be highly effective in rapidly reducing the HBV load and has a very high safety profile [4], although its long-term use has been jeopardised by the progressive appearance of viral resistance [10]. Dose-finding studies defined 100 mg per day as the dosage for patients with HBV-related chronic liver disease, one-third of the dose used in patients infected with HIV [4, 12]. In those studies, a negative serum HBV DNA result was the end-point, and no substantial differences were found between 25, 100, 300 or 600 mg, if not in the timing of serum viral clearance, which was slightly faster for the higher doses [4, 12]. However, the results of those studies, if evaluated with the current standards, are unsatisfactory due to the low sensitivity of the techniques used for the detection of HBV DNA in serum. In fact, the hybridisation techniques on liquid phase used in those studies had a detection limit of 1.5 pg/ml (approximately 84,000 IU/ml), which is far behind the current standard for the evaluation of an antiviral drug which is set at 25 IU/ml [13]. In doing so, the capacity of 100 mg daily of lamivudine to suppress

HBV replication was likely overestimated, especially if we consider the dose-dependent pharmacokinetics of the drug and therefore its serum concentration.

This study was carried out in the clinical practice and allowed us to evaluate, although in a non-randomised fashion, the effects of an initial high dose of lamivudine in patients with chronic HBV infection. Although this was a retrospective, non-randomised study, patients characteristics closely mirrored those commonly encountered in the everyday clinical practice in our geographic area, and patients' subgroups were well-balanced in terms of clinical and virological parameters. The main result of this study is that patients who received an initial high dose of lamivudine tended to have a lower rate of virological resistance (60% versus 76%) after an overall median duration of treatment of 45 months. Worth noting is that we observed that patients who received an initial high lamivudine dose had a significant delay in the appearance of drug resistance, with a median time to appearance of resistance of 48 months in the high dose group and 28 in the standard dose group. These results seem to suggest that the genetic barrier of lamivudine could be dose-dependent, and that higher-than-standard lamivudine dose may be able to delay the appearance of viral resistance. Honkoop et al showed a trend towards more profound suppression of viral replication with a lamivudine dose of 300 mg, suggesting further studies to define the degree of virus suppression required in clinical practice [14]. In a previous study, we demonstrated that it could be possible for large fluctuations in the lamivudine serum levels to be present in the earliest phase of treatment due to the binding of the drug to the wild type HBV polymerase molecules [15]. This might generate gaps of sub- or non-effective drug levels, which could be the cause of delayed or partial viral clearance. Moreover, the persistence of effective viral replication in the presence of fluctuating or sub-optimal drug levels could explain the high and early frequency of YMDD mutant selection. Thus, the initial high dose of lamivudine might fill this gap assuring effective drug levels and therefore preventing the early emergence of resistant strains.

**In conclusion**, this study showed that an initial high dose of lamivudine is able to significantly delay the appearance of resistance in chronic HBV patients. Although from the practical point of view this result may have little relevance in countries where other nucleos(t)ides are available for the initial treatment of chronic HBV patients, it might represent a financial advantage for countries with a high HBV endemicity and low economical resources. In this regard, it might be of interest to evaluate the outcome of patients treated with a

higher-than-standard dose of lamivudine in a prospective, randomised study carried out in these populations.

## Conflict of interest

No conflicts of interest exist.

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