Lamivudine therapy of chronic hepatitis B in three groups of patients: non-transplanted patients, liver recipients, and kidney recipients

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SUMMARY

Objective — As conflicting results have been observed by some authors in liver recipients, the aim of the study was to evaluate lamivudine therapy in 3 groups of patients with chronic hepatitis B: non-transplanted patients, liver and kidney recipients.

Methods — All patients were studied for clinical symptoms, hepatic enzymes, hepatitis B virus (HBV) serology, serum HBV DNA load, and HBV polymerase genotype (mutations associated with lamivudine resistance).

Results — During the 48-144 week-long follow-up (mean: 75 weeks), 23 non-transplanted patients, 5 liver and 6 kidney recipients were studied. A sustained biochemical and virological response was obtained in 19 out of the 23 non-transplanted patients and in 4 of 6 kidney recipients, while the 5 liver recipients did not respond. After the development of lamivudine resistance, mutations rtM204V and rtL180M were detected in all studied patients, mutation rtM207I in one, with similar results from traditional nucleotide sequencing and a commercial line probe assay.

Conclusion — The poor response to lamivudine in liver recipients requires further studies.

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Chronic hepatitis B is a potentially severe disease affecting 300 million people worldwide. Complications include cirrhosis and liver cancer [1]. Although the treatment of chronic hepatitis B is still imperfect, it is improving. Interferon-alpha induces the loss of the hepatitis B e antigen (HBeAg) and of hepatitis B virus (HBV) DNA (below 10^6 copies/mL) in 33% of patients [2]. Although this virological response is associated with improved liver histology and stops disease progression, interferon is not well tolerated [3]. On the other hand, lamivudine (2’-deoxy-3’-thiocytidine), an oral nucleoside analogue, has been registered for the treatment of hepatitis B and has been shown to be active and well tolerated [4].

However, as previously observed with this kind of antiviral treatment in human immunodeficiency virus infection, viral resistance to lamivudine has been reported, with an increase in HBV DNA titers and serum transaminases [5-9]. Resistance was associated with mutations in the conserved catalytic region of the HBV polymerase gene, especially in the tyrosine-methionine-aspartate-aspartate (YMDD) locus.

The aim of the present study was to retrospectively evaluate lamivudine therapy in 3 groups of patients: non-transplanted patients, liver recipients, and kidney transplanted patients.

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Patients and methods

Patients

Thirty-four patients were included in a retrospective, open, non-randomised study (table I). These chronic hepatitis B patients were treated with lamivudine and studied for 48 to 144 weeks (mean: 75 weeks). All patients were HBS antigen positive with biological, virological and histological signs of viral replication. All patients underwent liver biopsy before treatment. Transplanted patients were treated with lamivudine after transplantation.

Group A included 23 patients who did not receive graft. Eighteen had been previously treated with interferon-alpha, with no sustained response.

Group B included 5 patients, 3 with recurrent hepatitis B after liver transplantation for chronic hepatitis B, two of whom had received prophylactic treatment with HB Ig (hepatitis B immunoglobulins) with no sustained efficacy. HBV re-infection occurred after liver transplantation in the two other patients.

Group C included 6 kidney allograft recipients with chronic hepatitis B.

All patients were treated with lamivudine, 100 mg/day orally. Anamnestic information and a clinical examination were obtained every 3 months, as well as biochemical and virological data.

Conflicting results have been reported in liver recipients, and in our experience this group of patients seems to have a poor outcome.

Table I. – Clinical and biological characteristics before lamivudine therapy of 34 patients with chronic hepatitis B.

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<td>Mean HBV DNA (Meq/mL)</td>
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<td>Histological activity</td>
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<td>F: Fibrosis (0/1/2/3)</td>
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<td>F: Activity (0/1/2/3)</td>
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<td>(number and percent of patients)</td>
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**Laboratory tests**

Laboratory tests included serum alanine (ALT) and aspartate transaminases (AST); HBsAg, HBeAg and anti-HBe, tested with commercial immunoassays (Dade Behring, France), and HBV DNA with branched DNA signal amplification assay (Chiron, France) with a limit of detection of 0.7 × 10^6 genome equivalents (0.7 Meq) per mL (2.5 pg/mL).

When samples were available, virological resistance was studied by nucleotide sequencing. This was investigated when HBV DNA levels rose above 0.7 Meq/mL ("escape" sample) after an initial decrease below this limit during lamivudine treatment. Pre-treatment and escape samples were available for 5 of the 8 patients with lamivudine resistance. Sequencing was performed on an ABI Prism 377 automatic sequencer with dichlorohodamine-labeled dideoxynucleotides (Applied Biosystems Sequencing Analysis, Applied Biosystems, Perkin Elmer, Saint-Quentin, France). The sequences were analyzed using "Sequence Navigator" and "Sequence Navigator" programs (Applied Biosystems).

Sequences were compared for the mutations (new nomenclature, [10]): M204I (methionine-to-isoleucine substitution at codon 204), M204V (methionine-to-valine at codon 204), L180M (leucine-to-methionine substitution at codon 180), V/L/M207I (valine/leucine/methionine-to-isoleucine at codon 207) after amplification by nested-PCR with the following primers:

- **Internal primers:** sense 5'-TCGTCGATTGTCGACGCCTTAT-3' and antisense 5'-ACCCCATTTTGTGTTTATTG-3'

Results were controlled by a line probe assay (INNO-LiPA HBV DR, Innogenetics, Lille, France) according to the manufacturers' recommendations [11].

Liver histology before treatment was evaluated for activity and fibrosis according to international criteria [12].

Liver histology was repeated in liver recipients after lamivudine virological resistance appeared.

Responders to lamivudine treatment were defined as patients with a biochemical and virological response: ALT normalization and HBV DNA < 0.7 Meq/mL or 2.5 pg/mL.

**Statistical analysis**

Serum HBV DNA loads and ALT values were compared between liver and kidney recipients before treatment using the Mann-Whitney non parametric two-tailed test, with GraphPad Prism 2.0 software (GraphPad software, San Diego, CA). P values above 0.05 were considered to be non significant.

The comparison of response to lamivudine between liver and kidney recipients was obtained using the Fisher's exact test (Epilinfo6.04 - CDC and prevention, WHO, USA). The same test was used to compare liver and kidney recipients, for positivity or negativity of serum HBsAg before treatment and non response/"escape"/response to lamivudine, including time (duration of response) in the analysis.

**Results**

Lamivudine was well tolerated in the 3 groups of patients.

Group A: 22 (96%) of the 23 patients had showed a biochemical and virological response after an average of 20 weeks (range: 4-47) for ALT and 16 weeks (range: 4-47) for HBV DNA. In 3 responder patients (HBeAg-positive), an increase in HBV DNA was noted after 62 weeks (range: 12-84). However, ALT levels remained below pre-treatment values, and clinical status was satisfactory. At that time, genotyping showed mutations M204V, L180M and M207I in the tested patient. For the other 19 patients, response to lamivudine was sustained throughout follow-up (mean: 75 weeks). Serocconversion from HBeAg-positive/HBeAg-negative to HBeAg-negative/HBeAg-positive was observed in 4 out of 12 patients infected with wild-type HBV.

Group B (figure 1): biochemical and virological responses were first observed in 4 of the 5 patients, but HBV DNA increased again after an average of 44 weeks (range: 24-52). Mutations M204V and L180M were present in 2 of the 3 studied patients. In the third patient, an evaluation was only performed at the beginning of the HBV DNA increase and none of the mutations were detected. No mutations were observed in the neighbouring areas of the sequenced polymerase region, and the patient respected the treatment protocol. A second sample was collected from this patient one year later: HBV DNA load was higher, but stabilized with lamivudine, L180M and M204V mutations were present. Histological improvement was reported in 2 patients and stabilization in one, while the fourth worsened. Clinical improvement persisted in 3 patients. All 5 patients had positive serum HBeAg before treatment. No serocconversion were observed.

Group C: 5 of the 6 patients responded to lamivudine. One patient had an increase in HBV DNA load after 64 weeks, with viral DNA levels lower than before treatment, normal ALT and a satisfactory clinical status. This HBV strain carried the M204V and L180M mutations. The other 4 patients had a sustained response to lamivudine throughout follow-up. Serum HBeAg was...
positive in only 2 patients (one non responder, one responder) before treatment, and negative in the 4 other patients (3 responders, one “escape” patient).

The line probe assay showed the same mutations as sequencing. In addition, in 3 patients, mixed wild and variant strains at positions 180 (2 patients) and 207 (3rd patient) were detected after the development of lamivudine resistance. There were no differences in serum HBV DNA loads and ALT values between liver and kidney recipients before treatment (P = 0.66 for both HBV DNA and ALT). Despite the limited number of patients, there was a weak but significant difference between renal graft and liver recipients: relapse was more frequent in liver recipients (bilateral P = 0.047).

Moreover, in the 11 graft recipients, we compared non responders, “escape” patients and responders according to the positivity or negativity of serum HBeAg before treatment. Response to lamivudine appeared to be less efficient in positive HBeAg patients but this was not significant (P = 0.059). Six positive HBeAg patients had a non response or an “escape” response while only one negative HBeAg patient had an “escape” response. Three HBeAg-negative and one HBeAg-positive patient had a sustained response to lamivudine.

Discussion

Our results support others and show that lamivudine therapy improves virological and biochemical parameters in a high percentage of patients (91% of initial responders) [4, 11].

Among the 34 studied patients, 31 had an initial biochemical and virological improvement. However in the patient in group A who was not considered a responder, a decrease in ALT and HBV DNA titers to below pretreatment levels was noted. The non responder in group C had a transient biochemical response with ALT normalisation. A third patient in group B had only a decrease in HBV DNA concomitant with an increase in ALT levels; this patient was also considered a non responder (patient 1).

After one year of treatment, Lau et al. [13] showed that a loss of HBeAg and a sustained HBV DNA decrease only occurred in 17-33% of patients, and a loss of HBeAg followed by seroconversion to anti-HBe in 16-19%. In our study, the patients were investigated for a mean of 75 weeks, and 23 out of 34 (67%) had a sustained response (ALT and HBV DNA) that persisted throughout follow-up. Nevertheless, all 4 liver recipients who had an initial response relapsed after an average of 44 weeks. In group A, HBe seraconversion occurred in 4 out of 12 patients (33%) with wild-type HBV. Pretreatment ALT values were shown to be predictive of HBe seroconversion [14]. In our study also, the mean ALT level in group A before treatment was higher than in soroconverters (11 × upper limit of normal (ULN)) than in non soroconverters (2.5 × ULN).

The disadvantage of lamivudine therapy is the development of resistance in a high proportion of patients: 17% after 1 year, 26% after 2 years, and 49% after 3 years of therapy, as reported in studies from Asia; 32% after 1 year in a large trial from the United States [5, 7-9, 13, 15, 16]. Lock et al. found that lamivudine-resistant mutants were detected at similar rates in HBeAg-negative and in HBeAg-positive patients. In their study including 39 patients with HBeAg-negative chronic hepatitis B who had received lamivudine for at least 1 year, the rates of detection of lamivudine-resistant mutations after 1 and 2 years of treatment were respectively 10% and 56% [17]. Hadziyannis et al. obtained similar results with a virologic remission rate of 41.6% after 24 months among the 25 studied patients [18]. Twenty-nine percent of our initial responders showed resistance to lamivudine after a mean follow-up of 75 weeks.

Resistant mutations in HBV polymerase were investigated in 5 of the 8 patients who showed resistance by comparing nucleotide sequencing of the viral polymerase gene before treatment and after resistance. As expected, the M204V and L180M mutations were detected in all of them. The M207I mutation was observed in one of these 5 patients. The line probe assay detected mixed HBV strains showing a wild or a mutated codon at one position in the same sample for 3 patients. The progressive development of co-existing variant resistant strains and wild strains has been previously observed, and the line probe assay was shown to be more sensitive than traditional and more time-consuming nucleotide sequencing [11]. Thus, this assay could improve monitoring of antiviral treatments.

The long-term clinical significance of lamivudine resistance is a crucial issue. In liver recipients (group B) who initially responded to lamivudine, histological improvement or stabilisation was respectively reported in 2 and 1 patients, despite the increase in HBV DNA. Generally, the patients with virological “escape” to lamivudine maintained a satisfactory clinical status. In a large study in 258 Chinese patients with chronic hepatitis B, liver histology improved in 56% of patients receiving 100 mg of lamivudine daily, and YMDD mutations were not associated with
a decreased histological response [5]. Maintenance of low HBV loads and ALT levels thanks to lamivudine therapy could be beneficial despite virological resistance [19]. On the other hand, acute exacerbation and HBV clearance were described by Lian et al. after the development of YMDD mutations during lamivudine therapy [20]. Moreover, severe hepatitis has been reported 3 years after lamivudine withdrawal, even though a sustained favorable effect on hepatitis was observed by some authors after the end of the treatment [6, 16]. Thus, the long-term clinical significance of lamivudine resistance requires further analysis to confirm the possible benefit of continuing lamivudine in patients developing resistance.

An interesting finding in this study, despite the small size of the transplanted groups, was the high proportion of liver recipients who developed resistance to lamivudine, while the progression was comparable in kidney recipients and non-transplanted patients. Interestingly, mean HBV DNA loads and ALT levels before treatment were similar in liver and kidney recipients. All these patients received the same immunosuppressive treatment (ciclosporin) to protect them from graft rejection. However, before treatment, all 5 liver recipients showed positive serum HBeAg, whereas this only occurred in 2 (one non responder, one responder) of the 6 kidney recipients. Although this difference was not statistically significant, an enhanced response to lamivudine has been suggested by others in HBeAg-negative patients, and this point deserves further studies on additional patients [21]. Moreover, a sensitive quantitative PCR assay could be helpful in assessing virological response to treatment and clarifying the influence of pre-treatment HBe positivity. Furthermore, in liver transplanted patients, unlike kidney recipients, HBV recurrence occurs in virus-free hepatocytes, which could favor the appearance of mutant viruses.

In our study there were only 5 patients in the liver recipient group. Nevertheless, if a poor prognosis for evolution was confirmed in this population, especially in the presence of HBeAg positivity before treatment, these liver recipients would be good candidates for bitherapy for chronic hepatitis B. Conflicting observations have been reported in these cases. Shapira et al. concluded that lamivudine was beneficial and well tolerated in 3 children with HBV infection after liver transplantation [22]. Lamivudine was shown to be effective for the prevention of HBV-recurrence in low-risk liver recipients who were HBV-DNA negative before transplantation and had HB Ig treatment for at least 6 months without HBV recurrence [23]. However, other authors have noted that lamivudine resistance frequently occurs in patients with HBV infection after orthotopic liver transplantation and is associated with hepatic fibrosis and the necroinflammatory process [9, 21, 24]. Lamivudine resistance was detected in 13 out of 33 patients with post-liver transplantation hepatitis B, after a median of 61 weeks of treatment in a study by Fontana et al. [25]. A combination of antiviral therapies, with for example, lamivudine and adefovir dipivoxil might be necessary in these cases [13]. Walsh et al. reported a patient with fibrosing cholestatic hepatitis 15 months after liver transplantation for HBV-related cirrhosis, despite therapy with lamivudine and HB Ig. Addition of adefovir dipivoxil resulted in resolution of jaundice and normalization of liver biochemistry [26] suggesting that adefovir dipivoxil, lamivudine and HB Ig might be an effective antiviral strategy. Adefovir dipivoxil has indeed been shown to be a potent treatment for lamivudine-resistant HBV mutants as well as wild-type HBV [27]. Interferon-alpha was also effective for treating a flare-up of HBV infection after the emergence of a lamivudine-induced YMDD motif mutant [28]. Interferon-alpha combined with lamivudine was also useful in inhibiting virus replication in patients with lamivudine-resistant virus mutations [29]. Moreover, monitoring of virus load by sensitive PCR assays (detection level of around 10^3 HBV DNA copies/mL) could help predict lamivudine resistant HBV strains and allow a more rapid and efficient adaptation of antiviral treatments [30].

In a study in renal transplant patients, lamivudine was shown to be safe and effective in HBsAg-positive transplant recipients, with a rate of lamivudine resistance comparable to that of immunocompetent patients [31]. On the other hand, in a study by Han et al. in 6 HBsAg-positive renal allograft recipients treated with lamivudine for recurrent hepatitis B viremia, ALT normalization and HBV-DNA became undetectable, then reappeared later in 3 subjects, while preemptive or prophylactic lamivudine treatment in 10 patients proved to be more effective (only one patient with recurrent HBV viremia) [32]. Moreover, lamivudine was shown to be partially effective in preventing recurrent HBV infection when given before and after liver transplantation for chronic hepatitis B [32-34].

Thus, future studies are necessary to define the optimal prophylactic therapy in transplantation patients.

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REFERENCES


