Efficiency and safety of lamivudine therapy in patients with chronic HBV infection, dialysis or after kidney transplantation

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AIM: To analyze the effectiveness and safety of lamivudine treatment in patients with chronic HBV infection undergoing hemodialysis or after kidney transplantation, and to study the frequency of tyrosine - methionine - aspartate - aspartate (YMDD) mutation occurrence after lamivudine treatment.

METHODS: We analyzed 91 patients with chronic hepatitis B, among whom, 16 patients underwent hemodialysis, 7 patients had kidney transplantation and 68 patients had normal function of kidney. The hemodialysis patients were treated by lamivudine 300 mg/wk. Patients after kidney transplantation and patients with normal function of kidney were treated with lamivudine 100 mg/d. Therapy lasted for 12 mo. HBV-DNA, HBsAg, HBeAg and anti-HBe, and anti-HCV antibodies were assessed in sera of patients. The analysis was performed before and 6 mo after the end of lamivudine treatment. Before, during and after the lamivudine therapy, the number of erythrocytes, leukocytes, platelets and hemoglobin concentration, ALT and AST activity, as well as bilirubin, urea and creatinine concentrations were analyzed in sera from patients.

RESULTS: After the 12-mo lamivudine treatment, elimination of HBV - DNA was observed in 56% patients undergoing hemodialysis and in 53% patients with normal kidney function. Only 1 from 7 (14%) kidney-transplanted patients eliminated HBV-DNA. Furthermore, HBeAg elimination was observed in 36% hemodialysis patients, in 51% patients with normal function of kidneys and in 43% kidney-transplanted patients. Among the patients undergoing dialysis, no YMDD mutation was found after 12 mo of therapy, while it was detected in 9 patients (13%) with normal function of kidney and in 2 kidney-transplanted patients (29%, P<0.006). We did not observe significant side effects of lamivudine treatment in studied patients.

CONCLUSION: Effectiveness of lamivudine therapy in dialysis patients is comparable with that in patients with normal function of kidney. Lamivudine treatment is well tolerated and safe in patients with renal insufficiency undergoing hemodialysis and kidney-transplantation. However, in the latter group, high incidence of YMDD mutation after lamivudine treatment was observed.

INTRODUCTION

Patients undergoing hemodialysis and kidney transplantation are commonly endangered with an HBV infection, despite passive and active specific prophylaxis. One of the reasons of this state is insufficiency of immune response in such patients connected with chronic immune suppression. Among these patients, immune insufficiency influences the rate of chronic HBV, as well as HCV infection development[4].

Hemodialysis patients with an active chronic hepatitis B are usually disqualified for kidney transplantation. Moreover, active HBV infection in kidney-transplanted patients may influence the transplanted organ damage. This is due to the effect of deposition of immune complexes, composed of HBsAg, specific antibodies and acute phase proteins, in renal glomeruli[5]. The treatment aiming at preventing kidney transplant rejection can exert hepatotoxic effects and escalate HBV replication. The inhibition of HBV replication can diminish the danger of liver, kidney and pancreas damage[3].

Interferons are seldom used in antiviral therapy of HBV infection in dialysis or kidney-transplanted patients. Among these patients, the first choice of drugs is nucleotide analogues, mainly lamivudine[9].

Effectiveness and safety of lamivudine treatment in HBV-infected patients undergoing hemodialysis or kidney transplantation were studied. Results were compared to chronic hepatitis B patients with normal renal function. Moreover, the frequency of YMDD mutation after lamivudine treatment was analyzed.

MATERIALS AND METHODS

The study included 91 chronic hepatitis B patients treated with lamivudine. Among them 16 patients underwent hemodialysis (4 women and 12 men, aged 35-66 years), 7 received kidney transplantation (3 women and 4 men, aged 27-58 years) and 68 patients had normal renal function (21 women and 47 men, aged 18-78 years).

The kidney-transplanted patients as well as patients without kidney damage were treated with nucleotide analogue - lamivudine (Zeffix®, Glaxo Smith Kline, Great Britain) 100 mg daily. Patients undergoing hemodialysis received lamivudine 300 mg/wk: 100 mg after each dialysis (dialysis was performed three times a week). The duration of lamivudine treatment in all patients was 12 mo.
The occurrence of HBV-DNA, HBsAg, HBeAg and anti-HBe as well as anti-HCV antibodies in sera of the patients was assessed before and 6 mo after lamivudine treatment. The count of erythrocytes, leukocytes, platelets as well as concentration of hemoglobin, bilirubin, urea, creatinine and activity of ALT, AST were assessed before and after 1, 3, 12 mo of lamivudine treatment.

**Extraction of HBV DNA**

HBV-DNA was extracted from 200 µL sera of patients using a DNA isolation kit (Gene Elute Mammalian Genomic DNA Miniprep Kit, Sigma, USA). HVB-DNA was amplified by PCR with a pair of complement primers to genome (sense 5'-AG GGG AGG AGA TTA GGT TAA-3', antisense 5'-AGG AGT GCGAATCCACACTC-3') in 20 µL reaction mixture containing: 200 µmol/L dNTPs, 0.4 µmol/L all primers, 1.5 mmol/L MgCl₂, 1.0 U Taq polymerase (Sigma) and 4 µL DNA solution. Forty cycles of amplification were performed at 96 °C for 30 s, at 57 °C for 60 s and at 72 °C for 60 s. The products of amplification were detected by electrophoresis in 2% agar gel and stained with brom etidine. Electrophoretogram was visualized in a recording system and analyzed with a UVI-KS400i /Image PC system.

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The presence of HBs, HBe antigens as well as anti-HBe, anti-HCV antibodies was detected in sera by MEIA technique (ABBOTT, USA).

**Statistical analysis**

Statistical analysis was performed by use of chi - square test with Yates’s correction and non-parametric Mann - Whitney test. P<0.05 was considered statistically significant.

**RESULTS**

HBV and HCV coinfection was observed in 8 (50%) patients undergoing dialysis and in 3 (43%) kidney-transplanted patients. None of the chronic hepatitis B patients with normal renal function presented HCV infection.

**Table 1** Effectiveness of lamivudine treatment and frequency of YMDD mutation occurrence after lamivudine treatment in chronic hepatitis B patients: with proper renal function, undergoing dialysis and after kidney transplantation

| Patients | n of HBV-DNA (%) | Elimination of HBeAg (%) | Appearance Normalization of YMDD (%) | Activity (%) |
|----------|------------------|--------------------------|-------------------------------------|--------------|
| Normal   | 68               | 35 (53)                  | 91 (13)                             | 53 (79)      |
| Liver function |         |                          |                                     |              |
| Dialysis | 16               | 9 (56)                   | 6 (38)                              | 0 (0)        |
| After    | 7                | 1 (14)                   | 3 (43)                              | 2 (29)       |
| Kidney transplantation |        |                          |                                     |              |

¹P<0.02, χ² test with Yates’s correction, ²P<0.006, χ² test with Yates’s correction.

After a 12-mo lamivudine treatment, elimination of HBV-DNA was observed in (54/68) 79% patients with proper renal function, in (15/16) 94% patients undergoing hemodialysis and in all kidney-transplanted individuals (Table 2 and Figure 1).

We did not observe YMDD mutations in patients undergoing hemodialysis after 12-mo lamivudine treatment. However, this mutation was found in 9 (13%) patients with normal renal function and in 2 (29%) kidney-transplanted patients.

We did not observe any significant side effects of lamivudine treatment independent of renal insufficiency state. Moreover, no alterations were observed in morphology, total protein and albumin concentrations during lamivudine treatment (Table 2).

**DISCUSSION**

Treatment of chronic hepatitis B patients undergoing hemodialysis could bring additional obstacles in comparison to individuals with normal renal function[5]. This group of patients was characterized by a weak presentation of viral antigens on hepatocytes, which showed effect on the alterations of signal transduction to T cytotoxic lymphocytes[6]. Our investigations showed that the rate of HBeAg elimination in dialyzed patients was low in comparison to patients with proper renal function.

Serum lamivudine concentration was positively and linearly correlated with the level of renal insufficiency[8]. This enabled us to reduce the dosage of lamivudine in patients undergoing dialysis. In our observation, the lamivudine dose of 300 mg/wk was well tolerated and did not cause significant side effects. We did not observe significant side effects of lamivudine treatment in patients with proper renal function as well as in those with renal insufficiency. Park et al[7] presented similar observations concerning the safety and tolerability of lamivudine treatment in kidney-transplanted patients.

**Table 2** Mean of selected biochemical parameters before and after kidney transplantation

| Normal renal function | Dialysis | After kidney transplantation |
|-----------------------|----------|------------------------------|
| Alt (UL/L)            | 44.44    | 4.43                         | 3.63                     |
| Urea (mg/dL)          | 150      | 149                          | 15                        |
| Hemoglobin (mg/dL)    | 14.6     | 1.21                         | 1.21                      |
| Albumin (g/L)         | 3.75     | 4.2                          | 3.78                      |
| Urea (mg/dL)          | 30       | 140                          | 140                       |
| Creatine (mg/dL)      | 0.89     | 0.91                         | 8.05                      |

1 - Before therapy, 2 - After 12 mo treatment.

Figure 1 ALT activity in patients with chronic HBV infection treated with lamivudine.
Hepatocyte damage in the course of chronic hepatitis B was mainly immune mediated, in addition to a less significant role of direct HBV hepatotoxicity[6]. Immune suppression in kidney-transplanted patients could diminish specific anti-HBV responses, which could alter immune-mediated cytotoxic effects. This could result in the decrease of hepatic inflammation and low ALT activity. On the other hand, immune suppression could facilitate HBV replication. Large HBV viral load could cause different disorders such as accumulation of immune complexes in glomeruli, further indicating the necessity of antiviral therapy in these patients[9]. Treatment of patients with chronic HBV infection seemed to be necessary when the number of HBV copies exceeded 10⁵/mL, independent of ALT activity[10].

Besisik et al[11] showed a high frequency (50%) of YMDD mutation occurrence in chronic hepatitis B patients undergoing dialysis and in patients who did not receive antiviral treatment. Tang et al[12] described the appearance of YMDD mutation in chronic hepatitis B patients and kidney-transplanted patients after treatment with lamivudine. Until now many types of HBV mutations have not been described, such as, YMDD (Tyr-Met-Asp-Asp), YRDD (Tyr-Arg-Asp-Asp), YMDN (Tyr-Met-Asp-Asn). In some mutations partial or even full (YVDD - Tyr-Val-Asp-Asp) resistance towards antiviral treatment was observed[13]. Antiviral treatment resistance can also be caused by other factors not connected to a YMDD mutation. Fontaine et al[14] observed HBV viruses, without noticeable mutation but showing resistance towards lamivudine treatment in patients undergoing dialysis as well as in kidney-transplanted individuals. Currently there are only a few studies concerning the frequency of YMDD mutation occurrence in dialyzed patients after antiviral therapy. The interesting fact is that in our study we did not observe YMDD mutation in patients undergoing dialysis; hence the mutation rate was high in kidney-transplanted patients after lamivudine treatment.

Ben-Ari et al[15] described a significant decrease of HBV viral load in kidney-transplanted chronic hepatitis B patients after lamivudine treatment. Moreover, they observed elimination of HBeAg in 3 of 6 treated patients. Our investigations did not confirm such a good rate of lamivudine therapy response in kidney-transplanted patients. YMDD mutation incidence rate after lamivudine treatment was high in this group. It seems that elimination of HBV and not the decrease of HBV viral load made the treatment successful. Moreover, it is noticeable that elimination of HBeAg with ongoing minor HBV replication could suggest the formation of HBV mutants resistant to nucleotide analogue treatment.

In conclusion, treatment with lamivudine in dialyzed patients as well as in kidney-transplanted patients is well tolerated and safe. Effectiveness of lamivudine therapy in patients undergoing dialysis and in patients with normal renal function is comparable. The YMDD mutation rate in patients undergoing dialysis seems to be low after lamivudine treatment, while YMDD mutation is frequent in kidney-transplanted patients.

REFERENCES

1. Saha D, Agarwal SK. Hepatitis B and HIV infection during haemodialysis. J Indian Med Assoc 2003; 99: 194-199, 203,213
2. Martin E, Rendon P, De Diego L, Soria MJ, Martinez MC, Martin L. Role of lamivudine in the reactivation of hepatitis B virus infection in immunodepressed patients. Rev Esp Enferm Dig 2003; 95: 804-808, 799-803
3. Lau SC, Tse KC, Lai WM, Chiu MC. Use of prophylactic lamivudine and mycophenolate mofetil in renal transplant recipients with chronic hepatitis B infection. Pediatr Transplant 2003; 7: 376-380
4. Girndt M, Kohler H. Hepatitis B virus infection in hemodialysis patients. Semin Nephrol 2002; 22: 340-350
5. Meisel H, Preikschat P, Reinke P, Hocher B, Budde K, Bechstein WO, Neuhaus P, Kruger DH, Neumayer HH. Disappearance of hepatitis B virus core deletion mutants and successful combined kidney/liver transplantation in a patient treated with lamivudine. Transpl Int 1999; 12: 283-287
6. Johnson MA, Verpoorton GA, Daniel MJ, Plumb R, Mjv, Van Caesbroeck D, De Broe ME. Single dose pharmacokinetics of lamivudine in subjects with impaired renal function and the effect of haemodialysis. Br J Clin Pharmacol 1998; 46: 21–27
7. Park SK, Yang WS, Lee YS, Jung HH, Chang JW, Choi HJ, Han DJ, Park JS. Outcome of renal transplantation in hepatitis B surface antigen-positive patients after introduction of lamivudine. Nephrol Dial Transplant 2001; 16: 2222-2228
8. Zhang CP, Tian ZB, Liu XS, Zhao QX, Wu J, Liang YX. Effects of Zhaoyangwan on chronic hepatitis B and posthepatic cirrhosis. World J Gastroenterol 2004; 10: 295-298
9. Okamoto M, Omori Y, Kadotani Y, Ushigome H, Nakamura K, Akioka K, Yoshimura N. Renal transplantation in HBsAg- and HBV DNA-positive recipient: a case report. Transplant Proc 2003; 35: 286
10. Lok AS, McMahon BJ. Chronic hepatitis B. Hepatology 2001; 34: 1225-1241
11. Besisik F, Karaca C, Akyuz F, Horosanli S, Onel D, Badur S, Sever MS, Danaloglu A, Demir K, Kaymakoglu S, Cakaloglu Y, Okten A. Occult HBV infection and YMDD variants in hemodialysis patients with chronic HCV infection. J Hepatol 2003; 38: 506-510
12. Tang S, Ho SK, Moniri K, Lai KN, Chan TM. Efficacy of famciclovir in the treatment of lamivudine resistance related to an atypical hepatitis B virus mutant. Transplantation 2002; 73: 148-151
13. Ohishi W, Shirakawa H, Kawakami Y, Kimura S, Kamiyasu M, Tatsumi M, Tazuma S, Nakanishi T, Chayama K. Identification of rare polymerase variants of hepatitis B virus using a two-stage PCR with peptide nucleic acid clamping. J Med Virol 2004; 72: 558-565
14. Fontaine H, Thiers V, Chemet F, Zylberberg H, Poupon RE, Brechet C, Legendre C, Kreis H, Pol S. HBV genotypic resistance to lamivudine in kidney recipients and hemodialyzed patients. Transplantation 2000; 69: 2090–2094
15. Ben-Ari Z, Broida E, Kittiay C, Chagnac A, Tur-Kaspa R. An open-label study of lamivudine for chronic hepatitis B in six patients with chronic renal failure before and after kidney transplantation. Am J Gastroenterol 2000; 95: 3579-3583

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