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CASE REPORT

Successful treatment with tenofovir in a child C cirrhotic patient with lamivudine-resistant hepatitis B virus awaiting liver transplantation. Post-transplant results

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Summary

Antiviral treatment can be complex in decompensated hepatitis B virus (HBV) cirrhosis because of potential emergence of lamivudine-resistant mutants and worsening liver function, and to multifactorial nephrotoxicity. Negative HBV-DNA status by hybridization before liver transplantation is a favorable prognostic factor. We present the case of a 54-year-old HBV+ liver transplantation candidate who, after testing negative for HBV-DNA, developed YMDD lamivudine-resistant mutants resulting in a deteriorated clinical condition. After 8 months of adefovir plus lamivudine double therapy, only partial response was achieved. Tenofovir was added to this regimen, and an early decline of HBV-DNA was seen at 4 weeks without adverse events. The patient underwent transplantation. At 21-month postoperative follow-up, the patient's outcome was excellent. Post-transplantation HBV prophylaxis, taking into account the prior development of mutants, consists of hepatitis B immunoglobulin plus lamivudine and adefovir. Tenofovir was well tolerated and produced a fast antiviral response, suggesting its potential value in combined antiviral treatment for liver transplantation candidates.

Introduction

Liver transplantation (LT) is now performed in selected cases of hepatitis B virus (HBV) cirrhosis [1]. The prognosis of these patients has dramatically improved in recent years with the introduction of lamivudine and other antiviral agents [2,3]. At present, almost universal prevention of HBV recurrence with hepatitis B immunoglobulin (HBIg) and lamivudine post-LT has resulted in excellent patient and graft survival [4].

Lamivudine is orally administered and well tolerated, and these features make it an advantageous treatment as compared with interferon for patients with decompensated HBV cirrhosis [1]. Clinical use of lamivudine has been associated with rapid suppression of HBV-DNA to undetectable levels and improvements in biochemical, histological and clinical parameters. However, in cirrhotic patients this treatment must be indefinite and prolongation of lamivudine is associated with the emergence of resistant mutations and the risk of decompensated liver disease [5].

Lamivudine-resistant mutations in the YMDD motif in the C region of the HBV polymerase gene have been observed at a rate of 17%, 39% and 57% after 1, 2 and 3 years of treatment [6]. Appearance of lamivudine-resistant mutations before LT may be associated with a loss of clinical benefit. There is a higher risk of graft infection caused by less effective prophylactic therapy and HBIg is also rendered less effective, making combined antiviral treatment absolutely necessary [7].

In vitro data have suggested that adefovir should be effective against all types of lamivudine-resistant YMDD

mutant HBV strains [8]. However, has its clinical efficacy been reported only recently in chronic hepatitis B involving YMDD mutants (in both HBeAg-positive and HBeAg-negative patients) [9,10] and also in pre- and post-LT patients [11]. Administration of this drug is safe and well tolerated even in decompensated liver disease, and inhibition of viral replication is induced in a short time in most cases. Nevertheless, an associated risk of nephrotoxicity has been noted [11].

Tenofovir is currently approved for use in HIV-positive patients [12] and is usually administered in those with HBV coinfection It has been suggested as an option for severely ill patients with liver failure and a high risk of nephrotoxicity [13]. Recently, tenofovir has been shown to be an effective alternative to adefovir for the treatment of patients with lamivudine-resistant chronic hepatitis B infection [14] and for patients failing prior adefovir and lamivudine treatment [15].

We present the case of a patient awaiting LT who presented decompensated cirrhosis and complications during antiviral treatment. After a year of lamivudine monotherapy he developed YMDD mutants; adefovir was added with slow, partial response. HBV-DNA cleared and the patient became eligible for transplantation only after tenofovir was added to the antiviral regimen. Twenty-one months after transplantation the patient is HBV-negative and in good clinical condition.

Patient and methods

Virological methods

Hepatitis B, D, and C, and HIV serology were assessed by commercial immunoassays. Serum HBV-DNA was measured by a hybridization method (Hybrid Capture® System HBV DNA assay; Digene Corporation, Gaithesburg, MD, USA) with a sensitivity of 5 pg/ml. Representative stored serum samples were later studied by a quantitative real-time polymerase chain reaction (PCR) assay, using TaqMan probes (HBV PCR kit, Abbott Laboratories, Hamburg, Germany) in an ABI PRISM 7000 Sequence Detection Systems. This method has a detection range of 4 UI/ml to 3×10^7 UI/ml. Nucleic acid extraction was performed with the QIAamp DNA Blood Mini Kit, (Qiagen, Hilden, Germany).

Resistant mutants to lamivudine were studied by the Inno-LiPA HBV DR assay (Innogenetics N.V., Ghent, Belgium).

Case report

A man with no known allergies, ex-smoker and a moderate consumer of alcohol, was diagnosed in 1975 at the age of 26 with asymptomatic chronic hepatitis B infection. In 1996 he required hospitalization for abdominal pain and diarrhea. Abdominal CT showed superior mesenteric vein thrombosis that resolved spontaneously 1 year later. The study of thrombotic risk was negative. Laboratory analyses were normal except for mild liver enzyme alterations. Viral hepatitis serology was as follows: HBsAg positive, anti-HBs negative, anti-HBcIgM negative, anti-HBcIgG positive, HBeAg positive, antiHBe negative, HBV-DNA negative (by hybridization), anti-HCV negative, and anti-Delta negative. The patient was monitored on an outpatient basis with periodic abdominal ultrasound studies and laboratory analyses.

In 1998 analytical monitoring detected elevated transaminases [ALT 1.1 ukat/l (normal <0.73 ukat/l) and AST 1.34 ukat/l (normal <0.5 ukat/l)], thrombocytopenia at 43 000 cells/mm^3 and viral DNA at 144 ng/ml. The diagnosis of hepatic cirrhosis was established on radiological and analytical criteria. Abdominal ultrasound showed a small liver, morphologically consistent with chronic disease and no space occupying lesions. Portal vein diameter was increased and the patient presented splenomegaly without ascites. Interferon treatment was not contemplated because of the patient's thrombocytopenia and decompensated liver disease. In December 1998 the patient was admitted to the hospital for hepatic encephalopathy.

In March 1999 he started lamivudine at 100 mg daily within the program of compassionate use, with a favorable initial clinical and serological response. Viral DNA tested negative at the fourth month of treatment. The response persisted up to April 2000, at which time YMDD lamivudine-resistant mutants emerged. The patient experienced upper gastric tract hemorrhage secondary to grade II esophageal varices; Child B score was 7 points.

In June 2002 the patient was started on adefovir therapy at 10 mg/day combined with lamivudine, within the compassionate treatment program. The antiviral response was progressive but slow. HBV-DNA was still positive by hybridization after 8 months, and according to our hospital policy he could not be accepted as a liver transplant recipient. The patient presented ascites and a first episode of spontaneous bacterial peritonitis (SBP) related to *Streptococcus bovis*. He suffered hepatic encephalopathy and progressive worsening of Child-Pugh status.

In January 2003 he was classified as Child Pugh C. Additionally, the patient presented kidney failure and refractory ascites, which was treated with draining paracentesis and albumin. He could not be discharged home and remained in the hospital on the liver transplant waiting list. However, he was not eligible for transplantation because of detectable HBV-DNA. Laboratory tests on February 3, 2003 were as follows: creatinine 99 umol/l, urea 5.3 mmol/l, Na+ 135 mmol/l, K+

5.2 mmol/l, albumin 27 g/l, glucose 4.9 mmol/l, AST 1.05 ukat/l, ALT 0.5 ukat/l, total bilirubin 171 umol/l, GGT 0.27 ukat/l, alkaline phosphatase 1.7 ukat/l, leukocytes 2280 cells/mm^3 (N 54.5%, L 26.8%, M 13.6%), erythrocytes 2980×10^6/l, Hb 10.3 g/l, hematocrit 29.9%, Platelets 37 200 cells/mm^3, INR 2, fibrinogen 1.08 g/l.

The patient was started on tenofovir at 245 mg/day within the program of compassionate use on February 27, 2003. HBV-DNA measured 12 pg/ml and lamivudine-resistant mutations (L180M and M204V/I) were detected in serum. Administration of tenofovir while continuing with oral adefovir 10 mg daily and oral lamivudine 100 mg daily resulted in a fast decline of hepatitis B viremia out of the limit of detection by hybridation test in 4 weeks with negative results on March 24, 2003. At that time the patient was accepted as a liver transplant candidate. Table 1 shows the results of serology, ALT and DNA-HBV determinations.

The patient underwent transplantation on April 19, 2003 without complications. The donor was 42 years old and HBV markers were negative. The initial immunosuppressive treatment used was cyclosporine and basiliximab. The anti-HBV prophylactic regimen consisted of intramuscular HBIg and lamivudine plus adefovir.

Outcome after LT has been excellent. At 21 months the patient has an active, normal life and he has not needed additional hospitalizations. He is now receiving immunosuppressive treatment with cyclosporine and mycophenolate mofetil and anti-HBV prophylaxis. Liver enzymes are normal, HBsAg remains negative and anti-HBs levels are 200 IU/l. Analytical results in the most recent post-LT follow-up (December 2004) were as follows are: creatinine 127 umol/l, urea 6.3 mmol/l, albumin 46 g/l, glucose 5.7 mmol/l, AST 0.63 ukat/l, ALT 0.72 ukat/l, total bilirubin 28 umol/l, GGT 0.63 ukat/l, alkaline phosphatase 1.1 ukat/l.

Discussion

We describe a Child C cirrhotic patient with replicative HBV infection awaiting LT. The development of lamivudine-resistant mutants coincided with clinical worsening. He was treated with adefovir with slow, partial response and tenofovir was added because of clinical deterioration and development of kidney failure. HBV-DNA by hibridation cleared within 4 weeks and the patient was transplanted.

Combination therapy against hepatitis B, as is used in HIV infection, might be an effective pretransplantation therapeutic approach that would lessen the risk of drug resistance [16]. Some authors have suggested that combined adefovir plus lamivudine treatment might be a more appropriate antiviral strategy than lamivudine alone for waiting list patients [17,18].

At present, lamivudine and adefovir are the only antiviral agents approved for treating decompensated hepatitis B cirrhosis although several studies have confirmed that tenofovir is active against HBV wild-type and mutant variants, as well as HIV [14,15,18]. Recent pilot studies have shown significant activity of tenofovir against lamivudine-resistant HBV mutants in coinfected HIV-positive patients [19–22]. Tenofovir was the only drug that could be added to our patient's treatment, as other new antiviral agents, such as clevudine and entecavir are still in the stage of preclinical use [1].

Recent evidence has shown that tenofovir can be useful in patients with cirrhosis and severe liver failure [13,23] because of lamivudine-resistant mutations in the YMDD motif of the HBV polymerase gene. In one report, favorable results with tenofovir allowed a liver transplant candidate to be removed from the waiting list [24]. We are not aware of other published reports of tenofovir administration in patients awaiting LT, although a communication in the American Congress of Transplantation (Washington

Table 1. Analytical and therapeutical evolution before liver transplantation.

Date	1-12-98	11-3-99	14-4-00	1-2-01	9-5-01	24-8-01	28-5-02	5-6-02	21-6-02	13-1-03	3-2-03	27-2-03	24-3-03	31-3-03
ALT*	1.1	1.4	0.9	2.2	1.8	1.75	1.5	1.4	1.1	0.8	0.5	0.75	1.2	1.3
HBsAg	+	+	+	+	+	+	+	+	+	+	+	+	+	+
HBeAg	+	+	+	+	+	+	+	+	+	+	+	+	+	+
DNA-VBH ng/ml†	144	40	–	1039	577	674	591		66	13		12	–	
DNA-VBH UI/ml‡											20 241	15 914	771	315
YMDD Lam-R mutants			+	+					+			+		
Lamivudine 100 mg/day		Start	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes
Adefovir 10 mg/day								Start	Yes	Yes	Yes	Yes	Yes	Yes
Tenofovir 235 mg/day												Start	Yes	Yes

Notes: Liver transplantation in 19-4-2003.

*ALT normal value <0.73 ukat/l.

†DNA-HBV by hybridation method sensitivity of 5 pg/ml.

‡DNA-HBV by Tagman PCR sensitivity, 4 to 3×10^7 IU/ml.

2003) reported its use in a pilot study with eight post-transplantation patients [25]. The authors suggested tenofovir as an alternative to other antiviral agents for post-LT HBV prophylaxis because of its relative lack of nephrotoxicity [25].

Adefovir has been notably effective in conditions indicating a poor prognosis, such as fibrosing cholestatic hepatitis [26,27], and for the small number of major mutations in the HBV polymerase gene observed in recently published studies [28] after the third year of continuous treatment. This raises the possibility that long-term use of adefovir might not generate significant problems with drug-resistant strains [9,10,29].

Tenofovir may be a therapeutical alternative to adefovir [30]. In a recent study comparing adefovir and tenofovir in the treatment of lamivudine-resistant HBV infection some adefovir treated patients showed a weak antiviral response, whereas those receiving tenofovir had a stronger and earlier decline of HBV-DNA [14].

The interesting features of our case were the fast response, clinical tolerance and preservation of renal function, in addition to the persistent response after transplantation. However, most important was the fast antiviral response achieved, similar to that described by F. Van Bömmel [13,14], for two reasons: because the patient became eligible for transplantation and because of the favorable post-transplantation prognosis associated with viral DNA negative status. In the Consensus Conference (Geneva, Switzerland 2002) Hoofnagle underlined the importance of the initial antiviral response: early response may be a prognostic factor indicating persistent response [31].

Antiviral treatment should be considered in HBeAg-positive decompensated cirrhotic patients or HBeAg-negative chronic hepatitis patients with HBV-DNA levels of $>10^3$ copies/ml by PCR [17]. Lamivudine and adefovir, alone or in combination, offer efficacy, tolerance and safety in this group of patients [32]. Generally, the use of a third drug is not contemplated to accelerate the response or as an alternative therapy in cases of renal failure or potential mutations resistant to both antiviral agents.

Nevertheless, the emergence of drug-resistant HBV variants as a consequence of long-term antiviral therapy and the low rate of sustained, lasting response with existing therapies are important current problems. Extensive experience gained from treating HIV infection has shown that combination therapy is preferable to monotherapy [16,31]. The rationale for combination therapy for HBV infection has been reviewed and some authors strongly suggest the need for clinical trials investigating combined treatments especially in patients with decompensated disease [16,33]. Until these are available, individual reports and clinical experience can provide some indication as to where our efforts should be directed.

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