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BRIEF REPORT

Helga Meisel
Petra Preikschat
Petra Reinke
Berthold Hocher
Klemens Budde
Wolf Otto Bechstein
Peter Neuhaus
Detlev H. Krüger
Hans H. Neumayer

Disappearance of hepatitis B virus core deletion mutants and successful combined kidney/liver transplantation in a patient treated with lamivudine

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H. Meisel · P. Preikschat · D. Krüger
Department of Medical Virology, Charité, Humboldt Universität Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany

P. Reinke
Department of Nephrology and Intensive Care, Virchow Klinikum, Humboldt Universität Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany

B. Hocher · K. Budde · H. Neumayer (✉)
Department of Internal Medicine, Charité, Humboldt Universität Berlin, D-10098 Berlin, Germany
e-mail: hans-h.neumayer@charite.de,
Fax: + 49-30-2802-8471

W. Bechstein · P. Neuhaus
Department of Surgery, Virchow-Klinikum, Humboldt Universität Berlin, Augustenburger Platz 1, D-13353 Berlin, Germany

Abstract Hepatitis B virus (HBV) core deletion variants with enhanced viral replication are associated with rapid deterioration of liver function in renal allograft recipients. Antiviral agents such as famciclovir and lamivudine offer new treatment strategies for these patients. Appearance, accumulation and persistence of HBV core deletion mutants were closely monitored in a kidney transplant recipient with liver cirrhosis before and after initiation of antiviral treatment. Under treatment with famciclovir HBV DNA concentration decreased by 50%, HBV mutants persisted. After replacement of famciclovir by lamivudine HBV replication was reduced below the detection limit. Lamivudine was well tolerated and liver function improved. After successful combined kidney/liver transplantation the patient became HBsAg and HBV DNA (detected by PCR) negative under continuous hyperimmune globulin and lamivudine treatment. Antiviral therapy with lamivudine may be useful in treatment of progressive liver disease associated with HBV core deletion mutants in renal allograft recipients and may enable successful liver transplantation.

Key words Kidney transplantation · Hepatitis B core deletion mutants · Lamivudine · Famciclovir · Combined kidney-liver transplantation

Introduction

Chronic hepatitis B virus (HBV) infection in renal transplant recipients often leads to endstage liver disease [6]. Appearance, accumulation and persistence of HBV variants carrying deletions in the central part of the core (C) gene concomitant with virus mutations in the core promoter/enhancer II (Cp/Enh II) region correlate with the development of severe liver disease in renal transplant recipients [6, 7]. Most of these HBV mutants exhibit an enhanced replication and expression of core and polymerase proteins which may cause cell damage without immune attack, simply due to accumulation of aberrant virus proteins [6, 7]. Due to the high risk of re-infection, liver transplantation in patients suffering from highly replicative HBV infection is not rec-

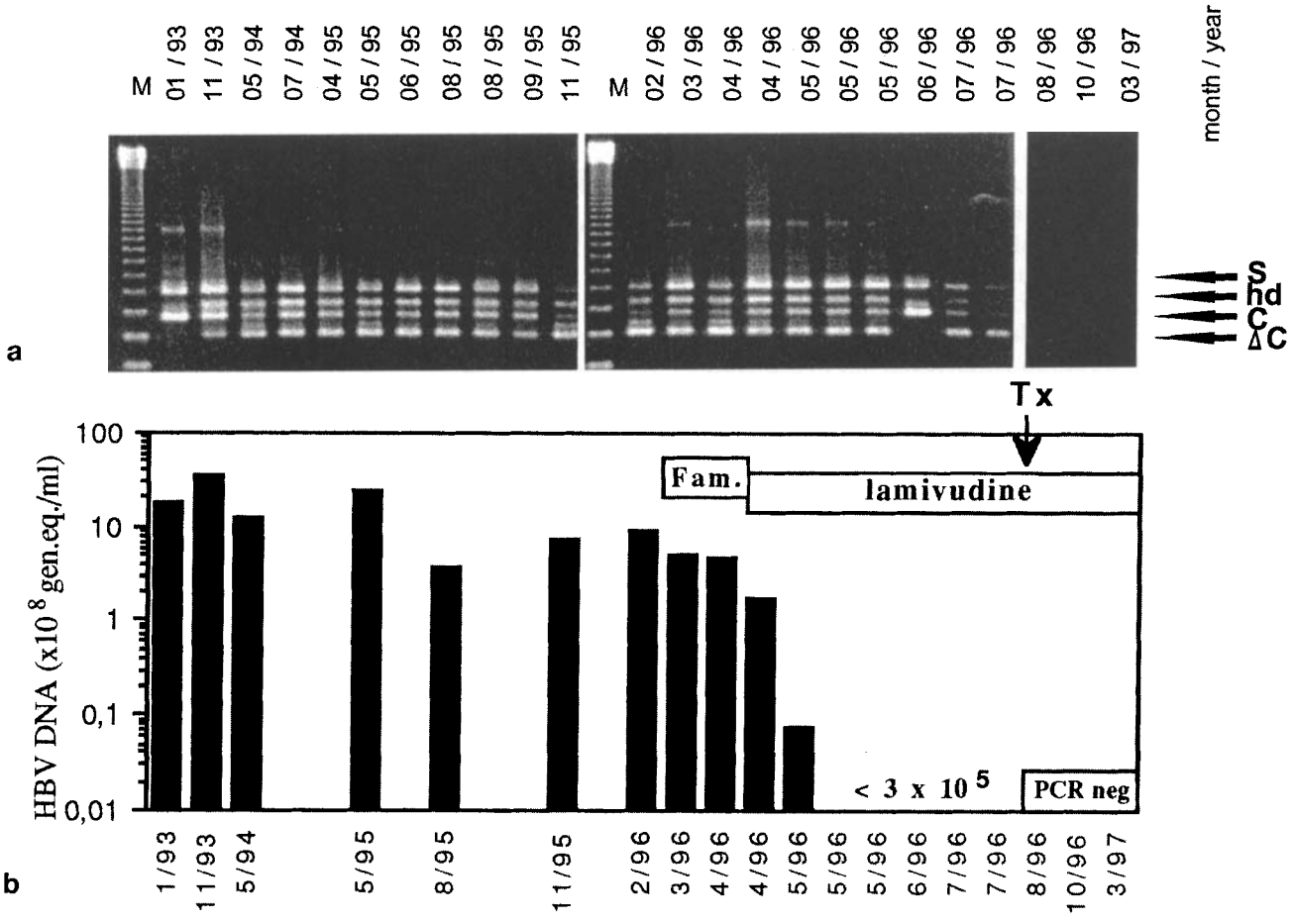

Fig. 1 **a** Agarose gel analysis of PCR products from HBV S and C genes in a 51 year old renal transplant recipient. *Arrows* mark the positions of S gene product *(S)*, C gene product *(C)*, shorter C-gene fragments *(ΔC)* as well as heteroduplices *(hd)* **b** Semiquantitative dot blot hybridization and PCR analysis of HBV DNA in sera (as described in ref. 1). *Fam.* Famciclovir; *Tx* combined kidney/liver transplantation; *gen. eq.* genome equivalents

ommended. Interferon alpha treatment, the only approved treatment for chronic HBV infection, is effective in fewer than 40% of patients, and may cause severe side effects in renal transplant patients. The high risk of severe rejection with concomitant graft loss and the poor antiviral long-term effect make interferon treatment in most patients after renal transplantation not advisable. So far, there are no viable treatment strategies for renal transplant recipients with HBV-induced liver cirrhosis.

Famciclovir, an oral form of penciclovir, is a new nucleoside analogue with antiviral activity against herpesviruses. Several reports described successful antiviral treatment of hepatitis B infection with famciclovir [8, 10, 11]. Early treatment of recurrent HBV infection after liver transplantation – i.e. immediately after detection of HBsAG in serum, but before development of recurrent graft hepatitis – with famciclovir was successful in the majority of patients in respect of lowering levels of detectable HBV-DNA [8]. During an observation period of about one year, a clinical improvement of recurrent graft hepatitis, as normalization of asparagine- and alanine transaminases (ASAT; ALAT), bilirubine levels and prothrombine time, and a decrease of HBV DNA levels below the detection limit of DNA hybridization, was observed in this study [8]. After initiation of famciclovir treatment, the conversion of recurrent delta positive hepatitis B after liver transplantation to seronegativity was recently reported [10]. Breakthrough infection during famciclovir therapy was shown to be associated with amino acid changes in the B-domain of the HBV polymerase [1].

Lamivudine, a cytosine nucleoside analogue, interferes with the reverse transcriptase activity of human immunodeficiency virus (HIV) as well as the reverse transcriptase of HBV [4]. Lamivudine has been shown to suppress effectively the HBV replication *in vitro* and *in vivo*. In a preliminary trial, HBV DNA became undetectable in all patients under treatment with lamivudine (100 mg/day), however, HBV DNA re-appeared after

cessation of therapy [5]. Lamivudine was also shown to be effective after liver transplantation, under long-term administration of the drug a complete (as determined by PCR) and sustained suppression of viral replication was observed [3]. In general, lamivudine is well tolerated, however, renal dysfunction may necessitate dose reduction. Recent reports describe the development of HBV resistance to lamivudine in patients after liver transplantation as well in immunocompetent patients [2, 9]. Lamivudine resistance is associated with HBV variants carrying mutations in the highly conserved YMDD motif of the viral transcriptase [2, 9].

To date, the successful use of these new compounds in antiviral treatment of renal transplant recipients with chronic hepatitis B leading to a transient or complete HBV elimination, has not been reported. Furthermore, successful antiviral treatment in patients with highly replicative HBV core deletion mutants has not been described yet.

Case report

Here we report on a 51 year old Caucasian male who acquired chronic HBV infection on maintenance hemodialysis between 1983 and 1985. After successful kidney transplantation in 5/1985, the immunosuppressive therapy consisted in administering prednisolone (10 mg/day) and azathioprine (75 mg/day). He tested positive for HBV DNA (by nested PCR; ref. 7), HBsAg, and HBeAg, however, markers for HCV, HDV and HIV were negative. Throughout the years the patient had a very high replication rate (mean HBV DNA level 1.5×10^9 genome equivalents (gen.eq.) / ml; Fig. 1) for HBV with moderately elevated serum transaminases. By sequencing at least 10 full-length HBV genomes from every serum sample, a total substitution of HBV wild-type by various variants carrying mutations in the Cp/Enh II region was observed from 11/1993 on (data not shown here).

The characterization of the patient's HBV genomes by C- and S-gene PCR is illustrated in Fig. 1. In addition to the Cp/Enh II mutations, deletions in the C gene appeared and accumulated in 1993. In 1994 an HBV variant with a central 30 amino acid deletion (aa 77–106; Preikschat et al., unpublished results) in the core protein became most prominent, representing 90% of the HBV population as determined by amplification and cloning of full-length HBV genomes and sequencing of the C gene from at least 10 genomes per serum sample (data not shown). In 1995 liver cirrhosis (Child A) was diagnosed on the basis of clinical, biochemical and ultrasonographic findings. The values for cholinesterase (CHE) and ALAT, as well as the serum creatinine concentrations from 1/1995 to 7/1996, are shown in Fig. 2. Due to steroid resistant rejection of the kidney graft tacrolimus (2 mg/day; trough levels: 5–8 ng/ml) was added in September 1995, however, graft function slowly further deteriorated during the following months (Fig. 2). After first ascitic decompensation and rapid deterioration of liver function (Child C: albumin 31.2 g/L, bilirubine 36.1 µmol/L, PT value 37% of normal, grade I encephalopathy) famciclovir treatment was initiated in March 1996 (250 mg b.i.d.) with the patients informed consent. Serum HBV DNA levels decreased by not more than 50% from 9×10^8 to 4.5×10^8 gen.eq./ml without change in the proportion of HBV mutants, no loss of HBV core deletion mutants could be observed (Fig. 1). Liver function did not improve. Famciclovir did not cause any notable side effects, renal function continued to decline slowly (Fig. 2).

Because HBV still remained highly replicative, famciclovir was replaced by lamivudine in April 1996 after 8 weeks of famciclovir treatment, again with the patients informed consent. Within 3 weeks after initiation of lamivudine (50 mg/day, adapted to renal function) HBV DNA decreased from 4.5×10^8 gen.eq./ml to levels below the detection limit of hybridization ($< 3 \times 10^5$ gen.eq./ml; Fig. 2). By PCR analysis we observed that the replication of HBV C gene deletion mutants was preferentially suppressed (Fig. 1; 6/96). An improvement of liver function and clinical state (from Child C to Child A: albumin 38.1 g/L, bilirubine 19.5 µmol/L, PT value in normal range, no encephalopathy, no ascites) was obvious after few weeks of therapy. Serum transaminases declined slowly, and CHE improved rapidly (Fig. 2). HBsAg and HBeAg remained positive, low levels of HBV DNA were detectable in serum by PCR only. Under treatment with lamivudine the patient experienced no side effects, and renal insufficiency further progressed steadily (Fig. 2).

Due to stabilization of clinical condition and effective suppression of HBV DNA replication, a successful combined liver/kidney transplantation could be performed in 7/96. The explanted liver showed a fine nodular cirrhosis with a moderate inflammatory response. HBV DNA could be detected in the explanted liver by HBV PCR (data not shown). After combined kidney/liver transplantation, the patient received high dose hyperimmune globulin therapy of anti-HBs (HBIg; Hepatect™, Biotest GmbH, Dreieich, Germany) in a dose of 10,000 units HBIg i.v. intraoperatively during the anhepatic phase and on the first postoperative day (POD). Because of insufficient neutralization of circulating HBsAg the patient received further daily doses of 2,000–8,000 units HBIg until HBsAg could no longer be detected on the seventh POD and an anti-HBs level of > 900 U/l could be achieved. Thereafter intermittent bolus doses of 2,000 units HBIg were administered to maintain an anti-HBs level of > 200 U/l. Concomitantly, the patient received lamivudine starting with a dose of 100 mg p.o. on the first POD, which was increased to 150 mg/day during the third post-operative week. Immunosuppression was started with Neoral (the microemulsion formulation of ciclosporine) in a dose of 4 mg/kg body weight in combination with mycophenolate mofetil in a dose of 1 g b.i.d. Steroids were rapidly tapered from 60 g prednisolone on POD1 to 15 mg by POD8. Cyclosporine doses targeted 200–300 ng/ml (monoclonal TDX assay) during the early postoperative period, and, after 12 weeks, at levels of 125–200 ng/ml.

Under this regime, both organs remained free from rejection. HBV DNA in serum and in peripheral mononuclear cells became undetectable by PCR (detection limit 10 genomes/ml) immediately after successful grafting. Additionally, HBsAg and HBeAg became negative in 8/96 (SORIN Biomedica ETI EBK 2, detection limit 0.2 PE units/ml). The patient was still negative for HBV DNA (by nested PCR) after 12 months of follow up (Fig. 1).

Discussion

Liver transplantation in patients with high viral replication is usually associated with a high risk of reinfection (> 50%) of the grafted liver, with rapidly progressive liver disease and poor graft and patient survival. The use of passive immunization with high-dose anti-HBs immunoglobulins (HBIg) lowers the rate of reinfection significantly. By using i.v. formulations such as the purified antibodies available in Germany (Hepatect™, Bio-

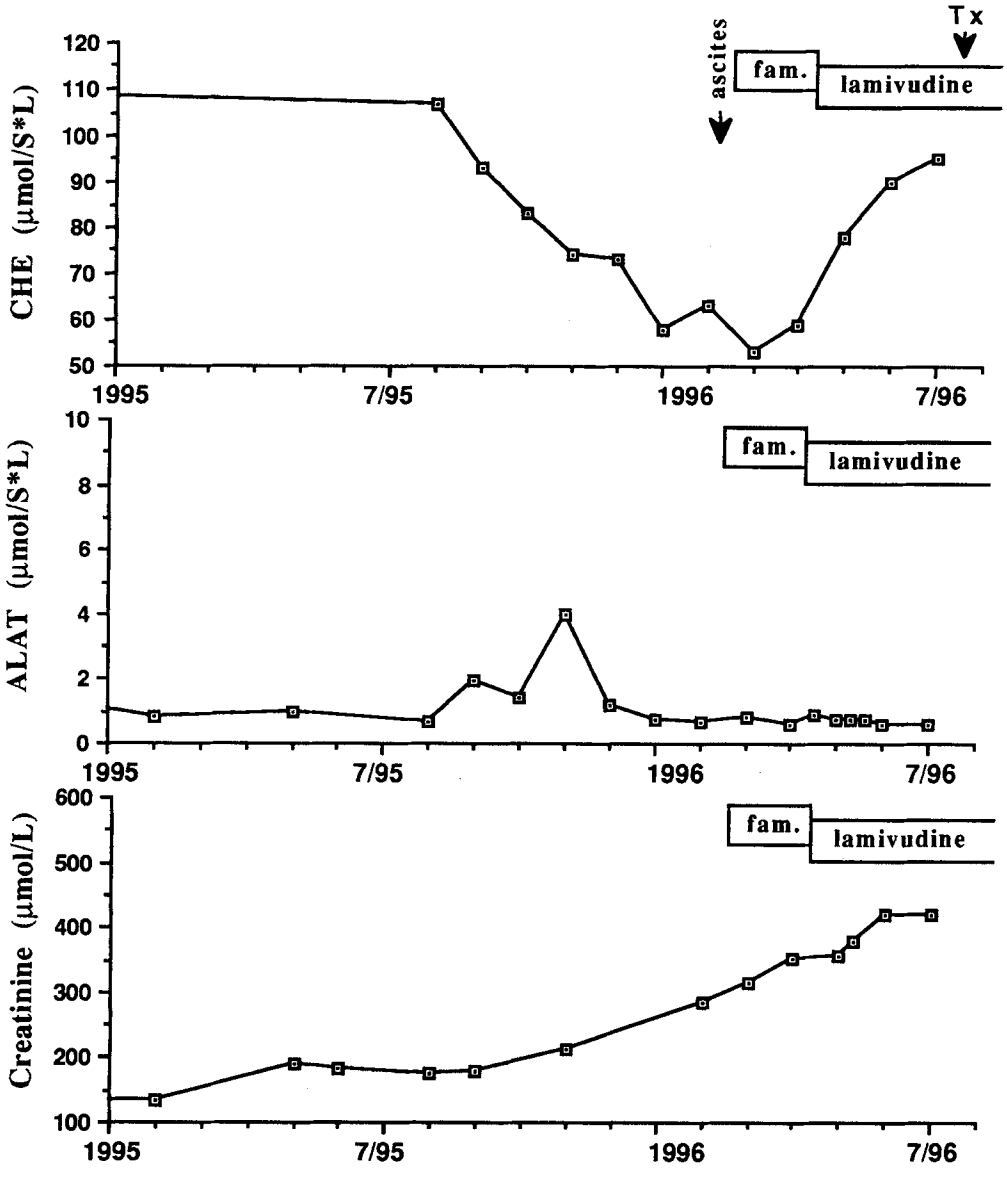

Fig. 2 Serum cholinesterase *(CHE)* values (normal > 90 µmol/S*L), alanine aminotransferase *(ALAT)* values (normal: < 0.8 µmol/S*L) and serum creatinine concentrations (normal: < 97 µmol/L) before and during antiviral treatment with famciclovir *(fam.)* and lamivudine in this patient. *Tx* Combined kidney/liver transplantation

test GmbH, Dreieich, Germany) this therapy has few, if any, side effects. However, this therapy is expensive and does not always prevent reinfection, especially in patients with highly replicative HBV infection before transplantation. Due to the history of steroid resistant rejection, interferon therapy would have had an unacceptably high risk of further deterioration of already poor renal function in our patient. Furthermore, due to the high replication rate of HBV mutants, interferon treatment may not have been successful. In this situation, new antiviral agents offered new treatment alternatives for this patient. Famciclovir and lamivudine were used in initial clinical studies; both of these new antiviral drugs are able to reduce serum HBV DNA concentrations rapidly [10, 4]. Extending recent reports, lamivudine, but not famciclovir, was effective in the treatment of progressive liver disease associated with the presence of HBV core deletion mutants in this long-term kidney graft recipient.

Although famciclovir was tolerated well, the drug could not significantly reduce the HBV DNA titers in serum and did not cause a substantial clinical improvement, both of them being preconditions for successful liver grafting. In general, using famciclovir treatment only 25–50 % of patients become HBV negative before trans-

plantation, although almost all patients respond to famciclovir treatment by reduction of their virus titers [8, 11].

Under lamivudine treatment we observed a rapid decline in HBV titers below the detection limit. In parallel, liver function and clinical condition improved substantially, thereby enabling successful liver grafting within three months. No side effects of lamivudine treatment were observed, especially the poor renal function remained stable. Despite the successful antiviral therapy with lamivudine, the patient still had very low amounts of HBV in the blood and in the liver before liver transplantation, probably due to the high replication rate of HBV variants before initiation of treatment. Similarly, under lamivudine treatment other authors could detect HBV DNA by PCR (but not by semiquantitative hybridization) in 12% of patients with high pre-treatment HBV DNA titers ($> 1.5 \times 10^6$ gen.eq./ml) whereas all patients with low replication numbers ($<1.5 \times 10^6$ gen. eq./ml) became negative in PCR after onset of lamivudine therapy [4].

The occurrence of lamivudine-resistant HBV mutants after 8–10 months of therapy [2, 9], was not observed in our patient after 12 months of follow up. The emergence of lamivudine resistant HBV may be facilitated by immunosuppression and may occur in 10–40% of patients [2, 9]. To date it is not clear whether such HBV mutants would aggravate the clinical course. High-dose HBIg therapy is an established effective therapy for prevention of HBV reinfection of the graft. In the high risk group with high replication measured by circulating HBV-DNA, preoperative antiviral therapy seems mandatory because of the higher failure rate of HBIg therapy in this subset of patients. Whether monotherapy with lamivudine or other antiviral agents is as effective in the long-term as HBIg is as yet undetermined. Indeed, one may speculate that the combination therapy of HBIg and lamivudine may lower the risk of selection of lamivudine resistant HBV mutations. For these reasons, we have continued to use HBIg in all patients with chronic HBV infection on a long-term basis. HBIg therapy in our center is only terminated upon reappearance of HBsAG in the serum. Under hyperimmune globulin therapy and continuous lamivudine treatment we observed effective suppression of HBV DNA and disappearance of HBV wild type, as well as HBV mutants, after liver transplantation. Whether lamivudine-resistant HBV variants will emerge in the future needs to be determined. To our knowledge, this is the first report on effective therapy of progressive liver disease associated with the presence of HBV core deletion mutants in a long-term immunosuppressed kidney transplant recipient.

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