

CASE REPORT

Successful treatment with adefovir dipivoxil in a patient with fibrosing cholestatic hepatitis and lamivudine resistant hepatitis B virus

K M Walsh, T Woodall, P Lamy, D G D Wight, S Bloor, G J M Alexander

Abstract

Fibrosing cholestatic hepatitis (FCH) is a severe clinical and histological variant of hepatitis B virus (HBV) infection seen most commonly in the HBV infected allograft after liver transplantation. Without treatment, FCH is fatal, rapidly and universally. Remission has been reported with lamivudine but is associated with evolving resistance to lamivudine. Adefovir dipivoxil has recently been reported to be a potent and highly effective inhibitor of HBV replication in both wild-type and lamivudine resistant HBV infection. We report a case of FCH 15 months after liver transplantation for HBV related cirrhosis despite therapy with lamivudine and hepatitis B immunoglobulin (HBIG). Within two weeks of commencing treatment with adefovir dipivoxil 10 mg once daily, the patient had made a remarkable recovery with resolution of jaundice and normalisation of liver biochemistry. HBV DNA and hepatitis B e antigen were lost from serum subsequently and liver histology had improved at four months. This case report suggests firstly, that advanced FCH can be reversed and secondly, that addition of adefovir dipivoxil to lamivudine and HBIG may be an effective antiviral strategy.

(Gut 2001;49:436-440)

Keywords: hepatitis B virus; fibrosing cholestatic hepatitis; adefovir dipivoxil; liver transplantation

Liver transplantation in patients with hepatitis B virus (HBV) infection is associated with a high rate of graft loss and poor survival following graft infection.¹ Long term use of high dose hepatitis B immunoglobulin (HBIG) alone reduces the risk of graft infection with HBV, but this protection is confined largely to those without evidence of active HBV replication at the time of transplant (both serum HBV DNA and hepatitis B e antigen (HBeAg) negative).² More recently, the nucleoside analogue lamivudine has been shown to reduce graft infection and damage with HBV^{3,4} but there is a significant rate of emerging resistance with long term therapy.⁵

Fibrosing cholestatic hepatitis (FCH) is a severe clinical and histological manifestation of HBV infection, seen most often in HBV infected recipients of liver allografts⁶ in which patients develop rapidly progressive liver failure. This has been attributed to direct cytopathic liver damage and is associated closely with widespread infection of hepatocytes with high intracellular concentrations of HBV antigens.⁷ These findings may be a direct effect of immunosuppressive agents on the virus⁸ as well as a reduced immune response to HBV.

Since the liver damage in FCH is cytopathic, inhibition of viral replication and reduced viral antigen expression should be beneficial. Isolated case reports suggest prolonged survival with ganciclovir⁹ or foscarnet¹⁰ but there was no evidence that either viral replication or liver damage were controlled. Most have found this approach wanting.¹¹ FCH has been treated successfully with the nucleoside analogue lamivudine^{12,13} but the high probability of emerging resistance is a serious concern.

Adefovir dipivoxil is the oral prodrug of adefovir, a phosphate nucleotide analogue of adenosine monophosphate, which inhibits viral polymerases and reverse transcriptases selectively and has broad spectrum antiviral activity against retroviruses, hepadnaviruses, and herpes viruses.¹⁴ Adefovir dipivoxil has been shown to be effective against lamivudine resistant strains of HBV in vitro¹⁵ and inhibitory for polymerases from lamivudine resistant mutants.¹⁶

We describe a patient who developed FCH despite therapy with HBIG and lamivudine and who responded to treatment with adefovir dipivoxil.

Abbreviations used in this paper: HBV, hepatitis B virus; HBIG, hepatitis B immunoglobulin; HBeAg, hepatitis B e antigen; FCH, fibrosing cholestatic hepatitis; HBsAg, hepatitis B surface antigen; HBcAg, hepatitis B core antigen; anti-HBe, antibody to hepatitis B e antigen; HDV, hepatitis D virus; anti-HBs, antibody to hepatitis B surface antigen; ALT, alanine aminotransferase; RT, reverse transcriptase.

Department of
Medicine, University
of Cambridge School
of Clinical Medicine,
Cambridge, UK
K M Walsh
T Woodall
G J M Alexander

Gilead Sciences Inc.,
California, USA
P Lamy

Department of
Histopathology,
Addenbrooke's NHS
Trust, Cambridge, UK
D G D Wight

Virco UK, Cambridge,
UK
S Bloor

Correspondence to:
Dr G Alexander, Box 157,
Addenbrooke's NHS Trust,
Hills Road, Cambridge
CB2 2QQ, UK.
gja1000@cam.ac.uk

Accepted for publication
19 December 2000

Case report

A 62 year old UK born Caucasian male and retired hospital porter, who had contracted HBV during his work, was referred in September 1997 because of hepatic encephalopathy, ascites, spontaneous bacterial peritonitis, and poor synthetic liver function. His serum was positive for hepatitis B surface antigen (HBsAg), HBeAg, HBV DNA, positive intermittently for IgM antibody to hepatitis B core antigen but negative for antibodies to hepatitis B e antigen (anti-HBe), anti-hepatitis D virus (HDV), and anti-hepatitis C virus. Liver biopsy confirmed cirrhosis with hepatitis B core antigen (HBcAg) staining of hepatocytes. The concentration of α fetoprotein was 46 IU/l (normal <10) but no focal lesion was seen on pretransplant imaging with ultrasound scan, computed tomography, or hepatic arteriography.

The patient underwent orthotopic liver transplantation without complications. Both donor and recipient were cytomegalovirus antibody negative. He was immunosuppressed initially with tacrolimus (target trough plasma level 5–15 μ g/l), azathioprine (1 mg/kg), and prednisolone 20 mg daily. Examination of the liver explant confirmed HBV related cirrhosis without hepatoma. Immunohistochemistry showed extensive cytoplasmic staining for HBsAg while 5–10% of hepatocytes showed nuclear and occasional cytoplasmic HBcAg staining.

The patient received 5000 IU of HBIg perioperatively and this dose was repeated three times during the first week after transplantation. Antibody titre to hepatitis B surface antigen (anti-HBs) at day 10 post-transplant was 777 IU/l. Thereafter, HBIg infusions were repeated to sustain anti-HBs levels above 100 IU/l. On day 21 post-transplant, serum was negative for HBV DNA and anti-HBs titre was 174 IU/l.

By day 28 he had become positive for HBV DNA in serum while also positive for anti-HBs at a titre of 124 IU/l. At day 42 post-transplant, despite continued HBIg administration, the anti-HBs titre remained persistently below 100 IU/l. At this point HBV DNA, HBeAg, and HBsAg were detected in serum but anti-HBe and anti-HDV were not. Liver biopsy at day 56 (fig 1) showed minimal portal inflammation without fibrosis. Immunohistochemistry for

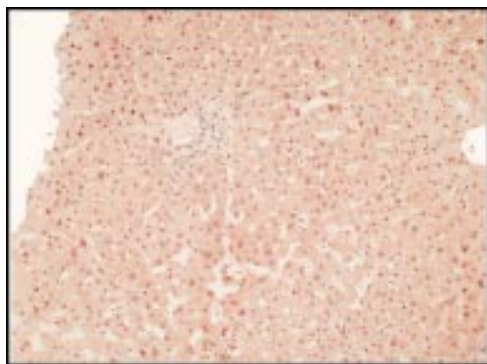


Figure 1 Liver biopsy on day 56 after transplantation, showing minimal portal inflammation without fibrosis.

both HBsAg and HBcAg was negative. He was commenced on lamivudine 100 mg.

At four months, liver biochemistry was normal apart from mildly raised alanine aminotransferase (ALT) at 61 IU/l (normal <55). HBV DNA, HBeAg, and HBsAg were present in serum. Anti-HBs titres remained <100 IU/l despite regular infusions of HBIg. Prednisolone was withdrawn while tacrolimus was continued to maintain a trough plasma level of 5–10 μ g/l, and lamivudine was continued at 100 mg daily.

At 10 months, serum albumin, alkaline phosphatase, and bilirubin were in the normal range but serum ALT had risen to 149 IU/l. HBV DNA, HBeAg, and HBsAg were present in serum. Repeat liver biopsy (fig 2A) showed progression to stage 2 liver fibrosis. Immunohistochemistry demonstrated that 30% of hepatocytes had strong nuclear and also cytoplasmic staining for HBcAg (fig 2B) while 50% of hepatocytes stained positively for HBsAg (fig 2C). Azathioprine was withdrawn; tacrolimus, lamivudine, and HBIg were continued as before.

At 15 months, he became unwell and developed jaundice with pruritus requiring hospitalisation. Liver biochemistry revealed albumin 34 g/l, bilirubin 94 μ mol/l (normal <17), alkaline phosphatase 171 IU/l (normal <130), and ALT 152 IU/l. Prothrombin time was 17 seconds (normal <16). HBV DNA, HBeAg, and HBsAg were present in serum. He was then demonstrated to have lamivudine resistant HBV infection.

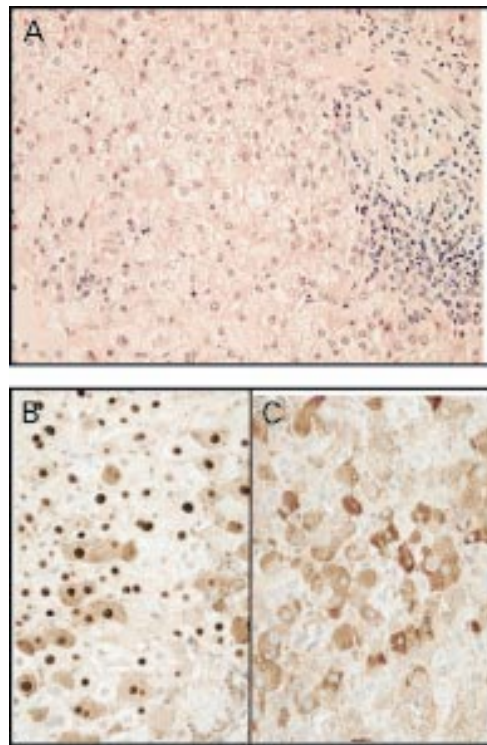


Figure 2 (A) Liver biopsy at 10 months after transplantation showing progression to stage 2 fibrosis. (B, C) Liver immunohistochemistry 10 months post-transplant demonstrating that 30% of hepatocytes had strong nuclear and cytoplasmic staining for hepatitis B core antigen (B) while 50% of hepatocytes stained positively for hepatitis B surface antigen.

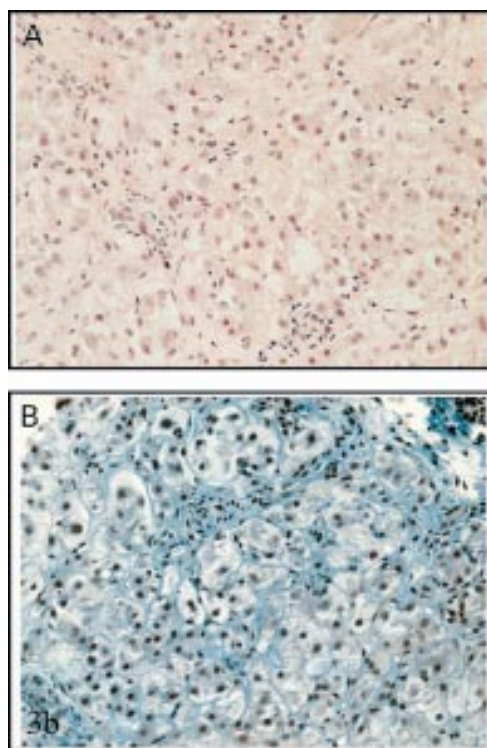


Figure 3 (A, B) Liver biopsy at 15 months post-transplant showing fibrosing cholestatic hepatitis.

Two groups of characteristic sequence mutations in the HBV polymerase gene are associated with lamivudine resistance¹⁷ and located in the reverse transcriptase (RT) domain of the protein, focused on the YMDD catalytic centre of the enzyme. In group I viruses the methionine residue at position 550 is substituted by valine; this change is associated with a second mutation of methionine for leucine at position 526. In group II viruses a mutation of the methionine 550 residue to isoleucine alone is responsible for resistance. Viral particles were isolated from serum and the region surrounding the RT active site domain was amplified using polymerase chain reaction. Fragments containing amino acids 347 to 690 were isolated and the sequence determined. The virus circulating in this patient showed the presence of the M550V and M526L combination.

Repeat biopsy (fig 3A) showed portal tract inflammation with occasional polymorphs, bile duct damage, and mild lobular lymphocytic and polymorphic infiltrate with feathery degeneration of hepatocytes. There was minor canalicular and cytoplasmic cholestasis but fibrous expansion of portal tracts with portal-portal linking fibrosis as well as isolation of small groups of hepatocytes by dissecting fibrous septae (fig 3B). Staining for HBsAg and HBcAg was positive, with the latter in both cytoplasm and nuclei. The overall appearances were characteristic of FCH.⁶

Adefovir dipivoxil was commenced at 10 mg daily while continuing lamivudine and HBIg as previously. Within two weeks he had made a remarkable recovery with resolution of all symptoms, and liver biochemistry and prothrombin time returned to normal (table 1).

Table 1 Decrease in serum levels of hepatitis B virus (HBV) DNA, bilirubin, and alanine aminotransferase (ALT) following treatment with adefovir dipivoxil (10 mg daily)

| Month | HBV DNA (copies/ml) | Bilirubin (mol/l) | ALT (U/l) |
|-------|---------------------|-------------------|-----------|
| 0 | 83 615 000 | 104 | 178 |
| 1 | 96 366 | 11 | 88 |
| 2 | 11 596 | 9 | 84 |
| 3 | 2 021 | 6 | 47 |
| 6 | <400 | 4 | 44 |

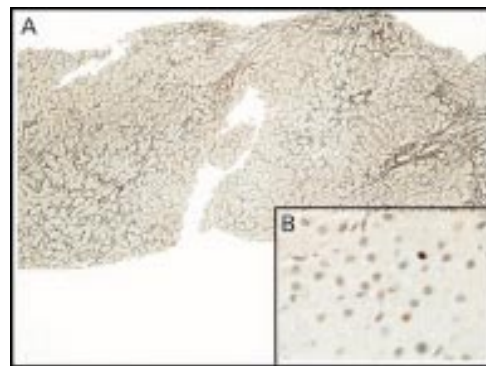


Figure 4 (A) Liver biopsy at day 102 after commencing adefovir dipivoxil 10 mg daily showing complete resolution of cholestasis, a marked decrease in inflammation, and regression of fibrosis (stage 2). (B) Liver immunohistochemistry at day 102 after commencing adefovir dipivoxil 10 mg daily demonstrating negative staining for hepatitis B surface antigen while hepatitis B core antigen was found in occasional hepatocyte nuclei but not in the cytoplasm.

HBV DNA concentration (Roche PCR) in serum fell rapidly and progressively to undetectable at day 106 after commencing adefovir dipivoxil (the limit of detection of the assay is 400 copies/ml). By day 138 post-adefovur dipivoxil, HBeAg/anti-HBe seroconversion had occurred. Repeat liver biopsy at day 102 post-adefovur dipivoxil showed complete resolution of cholestasis, a marked decrease in inflammation, and regression of fibrosis (fig 4A). There was occasional widening of portal tracts by fibrosis (stage 2) but no evidence of portal-portal bridging as before. Immunohistochemistry was negative for HBsAg while HBcAg was found in occasional hepatocyte nuclei but not in cytoplasm (fig 4B).

HBIg was withdrawn after 12 months of combination therapy. He has not experienced any side effects due to adefovir dipivoxil and remains well after 16 months of continuous treatment with adefovir dipivoxil and lamivudine.

Discussion

We believe that this is the first report of a patient developing FCH while being treated with lamivudine. The response to treatment of lamivudine resistant FCH with adefovir dipivoxil was rapid and complete and sustained at 16 months.

The risk of graft infection with HBV after liver transplantation is greater in those patients with detectable HBV replication at the time of transplantation. In the mid-1990s, many centres excluded patients with HBeAg or HBV DNA in serum from liver transplantation

because of the poor associated prognosis following graft infection with HBV. The Eurohеп consensus document in 1994 recommended that such patients should only undergo transplantation in the context of a clinical trial.¹⁸ For those patients without detectable HBV replication at the time of transplantation, the risk of graft infection and death from HBV related graft disease is reduced by HBIg.¹⁹

The introduction of lamivudine has revolutionised the management of patients undergoing liver transplantation for HBV infection, especially those with detectable HBV replication at the time of operation. Lamivudine can reduce the risk of graft infection with HBV after liver transplantation^{3,4} while the combination of HBIg and lamivudine is even more effective.²⁰ However, initial enthusiasm has been tempered by the recognition of evolving resistance to lamivudine with time, characterised by the YMDD mutations.⁵ Until now, the therapeutic options for managing lamivudine resistant HBV and related liver disease in the graft have been limited. A second transplant for HBV related graft failure carries a prohibitive HBV related mortality.²¹

Adefovir dipivoxil is an oral prodrug of adefovir whose active metabolite, adefovir diphosphate, inhibits viral polymerases at concentrations much lower than those needed to inhibit human DNA polymerases α , β , and γ .¹⁴ The intracellular half life of the active metabolite is approximately 30 hours, enabling once daily dosing. Adefovir dipivoxil has been shown to reduce HBV DNA levels by a median of 4.1 log₁₀ and induced seroconversion from HBeAg to anti-HBe in three of 15 (20%) patients who were treated for 12 weeks at 30 mg once daily.^{22,23} More importantly, adefovir dipivoxil has been shown to be effective in treating lamivudine resistant strains of hepatitis B^{14,15} and thus is an attractive option for patients with HBV graft infection, despite treatment with HBIg and lamivudine in combination.

A devastating form of cytopathic injury accompanies HBV infection in the graft, termed FCH,⁶ which untreated carries a mortality of 100%. The natural history of lamivudine resistant HBV infection in the graft and the speed of progress are less well documented but several deaths have now been reported.²⁴ Thus we believe that our patient had a very limited life expectancy. The introduction of adefovir dipivoxil was associated with rapid clinical, biochemical, virological, and histological remission. The persistence of HBV antigens in liver tissue, albeit at reduced quantities, despite control of HBV replication, reflects the fact that these agents do not eliminate HBV DNA from tissue and that transcription does continue. However, it is likely that infected hepatocytes will be eliminated with time and that HBV antigens would then be lost eventually. This assumes that there would be no loss of inhibition of HBV replication through failure of compliance or emerging resistance to the combination. To avoid

resistance, maximal suppression of viral replication is desirable. Combination therapy of nucleotide analogues with HBIg provides maximal suppression. However, the long term toxicity of the nucleotide analogues remains unknown at present.

We have recently added adefovir dipivoxil to lamivudine in four further patients with lamivudine resistant HBV graft infection, with uniform success, although follow up is limited. Preliminary experience in five patients with more benign disease suggests that this combination is likely to be effective.²⁵ In our patient described here, loss of HBV DNA from serum from such a high baseline level (by a decrease from baseline of >5.3 log₁₀) indicates the potency of the combination of lamivudine and adefovir dipivoxil, while seroconversion to anti-HBe is unique in our experience in this setting. Resistance to this combination has not yet been reported. Our experience with this combination may be a strong pointer to future therapy of HBV both within and outside the transplant setting.

- O'Grady JG, Smith HM, Davies SE, *et al.* Hepatitis B virus reinfection after orthotopic liver transplantation. Serological and clinical implications. *J Hepatol* 1992; 14:104-11.
- Samuel D, Bismuth A, Mathieu D, *et al.* Passive immunoprophylaxis after liver transplantation in HBsAg-positive patients. *Lancet* 1991;337:813-15.
- Grellier L, Mutimer D, Ahmed M, *et al.* Lamivudine prophylaxis against reinfection in liver transplantation for hepatitis B cirrhosis. *Lancet* 1996;348:1212-5.
- Bain VG, Kneteman NM, Ma MM, *et al.* Efficacy of lamivudine in chronic hepatitis B patients with active viral replication and decompensated cirrhosis undergoing liver transplantation. *Transplantation* 1996;62:1456-62.
- Ling R, Mutimer D, Ahmed M, *et al.* Selection of mutations in the hepatitis B virus polymerase during therapy of transplant recipients with lamivudine. *Hepatology* 1996;24:714-17.
- Davies SE, Portmann BC, O'Grady JG, *et al.* Hepatic histological findings after transplantation for chronic hepatitis B virus infection, including a unique pattern of fibrosing cholestatic hepatitis. *Hepatology* 1991;13:150-7.
- Lau JYN, Bain VG, Davies SE, *et al.* High-level expression of hepatitis B viral antigens in fibrosing cholestatic hepatitis. *Gastroenterology* 1992;102:956-62.
- Lau JYN, Bain YG, Smith HM, *et al.* Modulation of hepatitis B viral antigen expression by immunosuppressive drugs in primary hepatocyte culture. *Transplantation* 1992;53:894-8.
- Jamal H, Regenstien F, Farr G, *et al.* Prolonged survival in fibrosing cholestatic hepatitis with long-term ganciclovir therapy. *Am J Gastroenterol* 1996;91:1027-30.
- Angus P, Richards M, Bowden S, *et al.* Combination antiviral therapy controls severe post-liver transplant recurrence of hepatitis B virus infection. *Gastroenterol Hepatol* 1993;8:353-7.
- Yoshida EM, Wolber RA, Mahmood WA, *et al.* Attempted resolution of acute recurrent hepatitis B in a transplanted liver by the administration of ganciclovir. *Transplantation* 1994;58:956.
- Chan T-M, Wu P-C, Li F-K, *et al.* Treatment of fibrosing cholestatic hepatitis with lamivudine. *Gastroenterology* 1998;115:177-81.
- Faraidy KA, Yoshida EM, Davis GE, *et al.* Alteration of the dismal natural history of fibrosing cholestatic hepatitis secondary to hepatitis B virus with the use of lamivudine. *Transplantation* 1997;64:926-8.
- De Clercq E, Holy A, Rosenberg I, *et al.* A novel selective broad-spectrum anti-DNA virus agent. *Nature* 1986;323:464-7.
- Xiong X, Flores C, Yang H, *et al.* Mutations in hepatitis B DNA polymerase associated with resistance to lamivudine do not confer resistance to adefovir in vitro. *Hepatology* 1998;28:1669-73.
- Ono-Nita SK, Kato N, Shiratori Y, *et al.* Susceptibility of lamivudine-resistant hepatitis B virus to other reverse transcriptase inhibitors. *J Clin Invest* 1999;103:1635-40.
- Allen MI, Deslauriers M, Andrews CW, *et al.* Identification and characterization of mutations in hepatitis B virus resistant to lamivudine. *Hepatology* 1998;27:1670-7.

- 18 Muller R, Samuel D, Fassati LR, et al. "EUROHEP" consensus report on the management of liver transplantation for hepatitis B virus infection. European Concerted Action on Viral Hepatitis. *J Hepatol* 1994;21:1140-3.
- 19 Gugenheim J, Baldini E, Ouzan D, et al. Absence of initial viral replication and long-term high dose immunoglobulin administration improve results of hepatitis B virus recurrence prophylaxis after liver transplantation. *Transplant Proc* 1997;29:517-18.
- 20 Markowitz JS, Martin P, Conrad AJ, et al. Prophylaxis against hepatitis B recurrence following liver transplantation using combination lamivudine and hepatitis B immune globulin. *Hepatology* 1998;28:585-9.
- 21 Crippin J, Foster B, Carlen S, et al. Retransplantation in hepatitis B: a multicenter experience. *Transplantation* 1994; 57:823.
- 22 Jeffers L, Heathcote E, Wright T, et al. A phase II dose-ranging, placebo-controlled trial of adefovir dipivoxil for the treatment of chronic hepatitis B virus infection. *Antiviral Res* 1998;37:A197.
- 23 Heathcote EJ, Jeffers L, Wright T, et al. Loss of serum HBV DNA and HBeAg and seroconversion following short-term (12 weeks) adefovir dipivoxil therapy in chronic hepatitis B: Two placebo controlled phase II studies. *Hepatology* 1998; 28:317A.
- 24 Mutimer D, Pillay D, Dragon E, et al. High pretreatment serum hepatitis B titre predicts failure of lamivudine prophylaxis and graft re-infection after liver transplantation. *J Hepatol* 1999;30:715-21.
- 25 Perrillo R, Schiff E, Yoshida E, et al. Adefovir depovixil for the treatment of hepatitis B mutants. *Hepatology* 2000;32: 129-34.