CASE REPORT

Acute liver graft failure due to emergence of lamivudine resistant hepatitis B virus: rapid resolution during treatment with adefovir

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Abstract

Background—Strategies for prevention of liver graft reinfection by hepatitis B virus (HBV) have been developed during recent years. Initially, passive immunoprophylaxis with high titre HBV immunoglobulin (HBIG), followed by lamivudine prophylaxis, and then the combination of lamivudine and HBIG have been employed. However, suboptimal use of the combination may be associated with failure of prophylaxis reflected by the emergence of HBV species with genetic changes that confer resistance to lamivudine and HBIG. Reinfection of the graft by HBV can be associated with rapid development of liver failure.

Case report—A 43 year old HBV infected man received lamivudine before transplantation, and lamivudine and HBIG after transplantation. Despite prophylaxis, graft reinfection and severe hepatitis were observed. The observed serological evolution and genetic sequencing of the emergent HBV species suggested selection of lamivudine resistant and surface antigen escape mutants consecutively. Adefovir treatment began after the development of graft failure.

Outcome—A rapid exponential decline in serum HBV titre was observed. Liver function tests normalised and signs of liver failure resolved.

Conclusion—The use of HBIG and lamivudine permits prevention of graft reinfection by HBV for the majority of patients. Adefovir, a potent inhibitor of lamivudine resistant HBV, should be used when failure of prophylaxis is associated with graft hepatitis.

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Keywords: hepatitis B virus; adefovir; liver graft; lamivudine

Case report

A 43 year old Caucasian male underwent liver transplantation in April 1999. He had hepatitis B virus (HBV) induced liver failure with diuretic resistant ascites and recurrent variceal haemorrhage. He commenced treatment with lamivudine 150 mg/day in 1996, and treatment

continued until the time of liver transplantation. After a period of effective suppression, a secondary rise in serum HBV DNA was observed before transplantation, during 1998.

Liver transplantation was an uncomplicated procedure. Lamivudine was continued postoperatively, and hepatitis B immunoglobulin (HBIG) prophylaxis was also given. Repeated doses of HBIG were given to maintain serum antibody to hepatitis B surface antigen (anti-HBs) titres at a level greater than 100 IU/l. It was hoped that the combination of lamivudine and HBIG would prevent recurrence of HBV in the graft. Serum hepatitis B surface antigen (HBsAg) was undetectable until July 1999. Then, HBsAg and anti-HBs were simultaneously detectable in serum. There was ongoing treatment with HBIG although serum titre of anti-HBs steadily declined. At the time of HBsAg recurrence, liver function tests were normal. Biochemical liver dysfunction was first observed in November 1999 and persisted until the time of referral to the Birmingham Liver Unit in March 2000.

At that time, the following investigations were undertaken: liver function tests (alanine aminotransferase (ALT) 503 IU/l, aspartate aminotransferase (AST) 366 IU/l, alkaline phosphatase (ALP) 322 IU/l, bilirubin 52 µmol/l, albumin 43 g/l), electrolytes (creatinine 147 µmol/l), haematology (white blood cell count (WBC) 3.5, platelets 97, international normalised ratio (INR) 1.2), and serology (HBsAg +ve, anti-HBs undetectable, hepatitis B e antigen negative, antibody to hepatitis B e antigen positive, HBV DNA genomic titre >40 million copies/ml). Genetic sequencing of the viral polymerase gene confirmed that it encoded an isoleucine instead of methionine residue at position 552. Liver biopsy was performed and demonstrated chronic hepatitis with moderate inflammatory activity and ductular proliferation consistent with fibrosing

Abbreviations used in this paper: HBV, hepatitis B virus; HBIG, hepatitis B immunoglobulin; HBsAg, hepatitis B surface antigen; anti-HBs, antibody to hepatitis B surface antigen; HBcAg, hepatitis B core antigen; ALT, alanine aminotransferase; AST, aspartate aminotransferase; ALP, alkaline phosphatase; WBC, white blood cell count; INR, international normalised ratio; FCH, fibrosing cholestatic hepatitis; HIV, human immunodeficiency virus.

Figure 1 Liver biopsy performed in May, one month before hepatic decompensation and commencement of adefovir. Portal tracts are expanded by moderate chronic inflammation and fibrosis. There is widespread and strong expression of hepatitis B core antigen (immunoperoxidase stains brown) in hepatocyte nuclei and cytoplasm.

cholestatic hepatitis (FCH). Immunohistochemistry for hepatitis B core antigen (HBcAg) was strongly positive in the nucleus and cytoplasm of most hepatocytes (see fig 1). During the following four weeks the patient developed graft failure with ascites and jaundice. Investigations at that time, prior to commencement of adefovir (see fig 2), revealed the following: liver function tests (ALT 488 IU/l, AST 557 IU/l, ALP 260 IU/l, bilirubin 260 μ mol/l, albumin 33 g/l), electrolytes (creatinine 160 μ mol/l), haematology (WBC 2.3, platelets 134, INR 1.7), and serology (HBV DNA titre 9 million copies/ml). Treatment with adefovir (10 mg/day) was added to lamivudine 100 mg/day. Subsequently, during two months of follow up, liver failure resolved with disappearance of ascites and jaundice. Serum HBV DNA titre declined by more than 4 $\log_{10}$, and treatment with both drugs is ongoing. At the time that adefovir was commenced, trough blood cyclosporin level was high and appropriate dose reduction was made.

Discussion

Successful long term outcome following liver transplantation for HBV requires an effective strategy to prevent graft reinfection. The first effective strategy required the administration of high titre HBIG. Passive immunoprophylaxis usually prevented graft reinfection when transplantation was performed for patients with low levels of viral replication. However, passive immunoprophylaxis was often unsuccessful when transplantation was undertaken for patients with high levels of replication.¹ Thus although serum HBsAg negativity could be sustained for a variable period following transplantation, graft reinfection and recurrent HBsAg'aemia typically ensued during the first post-transplant year. At the time of this recurrence, HBIG may select as the dominant HBV species virus with specific amino acid substitutions in the immunodominant "a" determinant of HBsAg (a so-called "surface antigen escape mutant").²⁻⁴

Lamivudine is a potent inhibitor of HBV replication and now has an established role for the prevention of graft reinfection following liver transplantation. Published experience confirms that lamivudine monotherapy, given before and after liver transplantation, can prevent significant graft reinfection for selected patients.⁵ Unfortunately however, graft reinfection can occur despite lamivudine prophylaxis, and is associated with selection and then emergence of viral species with specific mutations in the polymerase gene. These changes comprise a methionine for valine (M552V) or methionine for isoleucine (M552I) substitution at amino acid residue 552, with other changes including methionine for leucine at position 528 (L528M) of the polymerase. Indeed, these lamivudine resistant species were first described in the context of failed prophylaxis following liver transplantation.⁶ As observed for

Figure 2 Serum bilirubin, alanine aminotransferase (ALT), and hepatitis B virus (HBV) DNA measured before and during adefovir treatment. HBV DNA was measured with the Roche Amplicor HBV Monitor assay (Roche Molecular Systems, Inc., Pleasanton, California, USA). The lower limit of detection of the assay is 400 genomic copies/ml (shown by the broken line).

HBIG prophylaxis, failure of lamivudine prophylaxis may be predicted for patients with high pretreatment serum HBV titre.⁷

Currently, prophylaxis protocols employing both HBIG and lamivudine have been adopted by most units, and HBV relapse in this context is rare.⁸ However, our patient relapsed despite the use of lamivudine before, and lamivudine and HBIG post-transplantation. We believe that failure of prophylaxis is a consequence of the emergence of lamivudine resistant virus before transplantation. He commenced lamivudine in 1996, three years before transplantation. Although not confirmed by genetic sequencing, reappearance of serum HBV DNA in 1998 after two years of suppression is strongly suggestive of the emergence of resistant species. It is recognised that resistant species emerge in the majority of patients during long term therapy.⁹ Resistant HBV species seldom emerge during the initial six months of lamivudine therapy.¹⁰ We believe therefore that successful strategies that use lamivudine pretransplantation must demand that transplantation is undertaken within six months of commencement of lamivudine, thus pre-empting the emergence of drug resistant species.

Thus in the absence of ongoing suppression by lamivudine, HBIG constituted the mainstay of post-transplant prophylaxis for our patient. Simultaneous detection of both HBsAg and high titre anti-HBs in his serum during HBIG therapy suggests selection of an "escape mutant" at the time of HBIG failure. Subsequently, despite ongoing administration of HBIG, serum titre of anti-HBs declined, and then biochemical liver dysfunction ensued.

In the context of immunosuppression, HBV infection can cause aggressive hepatitis and subacute liver failure. The histological appearance associated with this type of liver failure can be distinctive, frequently referred to as fibrosing cholestatic hepatitis (FCH).^{11,12} FCH has been observed in the graft after liver transplantation, and has also been observed in the liver of other immunosuppressed patients.¹³ Early reports of this condition highlighted the almost universal fatal outcome. It has been suggested that liver failure associated with FCH may be due to massive viral antigen expression associated with extremely high levels of viral replication. Our patient developed liver failure associated with the histological changes of FCH and with very high levels of viral replication (reflected by the high serum HBV titre and by immunohistology). In the absence of prompt effective treatment, we predicted a very poor prognosis. Effective treatment required prompt and potent inhibition of viral replication. Acute liver failure¹⁴ and FCH¹³ in immunosuppressed patients has been successfully treated with lamivudine. However, genetic sequencing confirmed that our patient had FCH due to lamivudine resistant virus. Previously, we have reported four patients with FCH and graft failure due to lamivudine resistant virus.¹⁵ Three died, and one patient underwent retransplantation. For those four patients, sustained and effective inhibition of replication was not achieved with high dose lamivudine, ganciclovir, or famciclovir treatment. Indeed, clinical cross resistance between

lamivudine and famciclovir is well documented.¹⁶

Adefovir dipivoxil is the oral prodrug of an acyclic nucleotide monophosphate analogue. It has a broad spectrum of antiviral activity against retroviruses, hepadnaviruses, and herpesviruses. Treatment with adefovir of wild-type HBV in non-immunosuppressed patients effects potent inhibition of replication and rapid decline of serum HBV titre.^{17,18} A median 4 log₁₀ decline in viral titre was observed during a treatment period of 12 weeks. A potential role of adefovir for the treatment of lamivudine resistant virus has been suggested by in vitro studies.¹⁹⁻²¹ In vitro, adefovir retains activity against HBV polymerase containing lamivudine resistance mutations. In the case of human immunodeficiency virus (HIV), reduced susceptibility to adefovir is conferred by the K65R mutation of the reverse transcriptase which has no homologue in HBV polymerase. However, in HIV, this resistance is attenuated by the lamivudine resistance M184V mutation (homologue of the M552V mutation of HBV polymerase). Indeed the M184V mutation leads to adefovir hypersensitivity in HIV-1. There is therefore a virological precedent for using adefovir against lamivudine resistant HBV species. Recently, Perrillo *et al* reported successful treatment of five patients with lamivudine resistant HBV infection with adefovir.²²

Using a sensitive polymerase chain reaction based assay with a broad detection range, we demonstrated a 4 log reduction in serum HBV titre over a two month treatment period. Ongoing decline in serum titre suggests that further decline will be observed. Inhibition of replication has been associated with prompt improvement of liver function tests and recovery from liver failure.

In summary, this patient demonstrates some problems and potential solutions for prevention and treatment of graft infection by HBV. Prolonged pre-transplant treatment with lamivudine permits emergence of resistant species with resumed viral replication at the time of transplantation, and should be avoided. High levels of replication at the time of transplantation permits the emergence of HBsAg escape mutants during HBIG therapy. As predicted by in vitro studies, adefovir inhibits replication of lamivudine resistant virus.

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