

Postliver transplant allograft reinfection with a lamivudine-resistant strain of hepatitis B virus: Long term follow-up

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EM Yoshida, MM Ma, JE Davis, et al. Postliver transplant allograft reinfection with a lamivudine-resistant strain of hepatitis B virus: Long term follow-up. Can J Gastroenterol 1998;12(2): 125-129. Lamivudine is a nucleoside analogue with efficacy in the suppression of hepatitis B viral (HBV) replication. In a previously reported study, lamivudine was administered to patients with chronic, actively replicating HBV infection who subsequently underwent liver transplantation. Patients became serum HBV DNA-negative in response to lamivudine before transplantation, which was continued in the post-transplant period. Two of four patients surviving the immediate postoperative period developed allograft reinfection 240 and 409 days post-transplant. The strain of the re-infecting virus was analyzed, and a mutation in the YMDD region of the viral polymerase conferring resistance to lamivudine was discovered. The long term follow-up of these two patients is reported. The first patient developed ascites 16.5 months after allograft reinfection. A transjugular liver biopsy performed 18 months after the emergence of the lamivudine-resistant strain revealed cirrhosis and lobular hepatitis without rejection. The gradient between hepatic vein wedged and free pressures was 13 mmHg, consistent with portal hypertension. The second patient, 16 months after allograft reinfection with the lamivudine-resistant strain, is without clinical evidence of portal hypertension, although liver enzymes remain elevated. Both patients were given a trial of famciclovir, which did not significantly suppress HBV viremia. In conclusion, lamivudine-resistant HBV strains

with the YMDD mutation may have an aggressive clinical course with rapid progression to cirrhosis. Famciclovir did not appear to be an effective rescue agent in these two patients.

Key Words: *Allograft, Cirrhosis, Hepatitis B, Lamivudine resistance, Liver, Reinfection, Transplantation, YMDD mutation*

Suivi à long terme d'une récurrence d'infection post-transplantation hépatique par une souche du virus de l'hépatite B résistante à la lamivudine

RÉSUMÉ : La lamivudine est un analogue des nucléosides qui supprime efficacement la réplication virale du virus de l'hépatite B (HBV). Dans le cadre d'une étude publiée antérieurement, la lamivudine a été administrée à des patients atteints d'une infection à HBV chronique et active qui ont par la suite subi une transplantation hépatique. Les patients sont devenus séronégatifs à l'égard de l'ADN de l'HBV en réponse à la lamivudine avant la transplantation. Ce traitement a été poursuivi après la transplantation. Deux patients sur quatre ayant survécu à la période postopératoire immédiate ont développé une nouvelle infection de l'allogreffe 240 et 409 jours après l'intervention. La souche du virus a été analysée et on a pu imputer la résistance à la lamivudine à une mutation de la région YMDD de la polymérase virale. Le suivi à long terme de ces deux patients est présenté ici. Le premier a développé de l'ascite 16,5 mois après la nouvelle infection de l'allogreffe; une biopsie hépatique trans-jugulaire effectuée 18 mois

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après l'émergence de la souche résistante à la lamivudine a révélé une cirrhose et une hépatite lobulaire sans rejet. Le gradient entre pression hépatique bloquée et libre a été de 13 mm Hg, concordant avec une hypertension portale. Six mois après la réinfection de l'allogreffe par la souche résistante à la lamivudine, le deuxième patient ne présente aucun signe clinique d'hypertension portale, bien que ses enzymes hépatiques

restent élevées. Les deux patients ont reçu à l'essai du famciclovir, qui n'a pas significativement supprimé la virémie à HBV. En conclusion, des souches d'HBV porteuses de la mutation YMDD résistantes à la lamivudine peuvent provoquer une forte évolution clinique, avec progression rapide vers la cirrhose. Le famciclovir n'a pas semblé d'un grand secours chez ces deux patients.

Until recently, patients with chronic hepatitis B virus (HBV) infection were not considered candidates for liver transplantation because of the high risk of allograft reinfection (1) and poor clinical outcome – 48% five-year survival versus 77% for primary biliary cirrhosis (2). The use of high dose parenteral HBV immune globulin (HBIG) as prophylaxis against reinfection (3-5) can reduce this risk to a clinically acceptable level, but the economic costs of such a prophylactic program are considerable. It is obvious that an effective form of anti-HBV prophylaxis that does not require HBIG would result in significant cost savings and allow more patients with HBV infection to be offered transplantation.

Lamivudine is a nucleoside analogue that is an effective antiretroviral agent in the treatment of human immunodeficiency virus (HIV) infection (6-8). Lamivudine was initially discovered to be effective in the suppression of active HBV infection in those co-infected with HIV (9,10). Later clinical trials in the non-HIV setting have confirmed the efficacy of lamivudine in suppressing HBV replication (11,12). Lamivudine appears to be a logical choice for prophylaxis against allograft reinfection in the transplant setting. A pilot study in liver transplantation with lamivudine as primary prophylaxis was undertaken at the University of Alberta (UA). It was reported that two of four long term transplant survivors developed allograft reinfection while on lamivudine (13). The reinfecting virus was sequenced and found to contain a point mutation in a specific locus of the polymerase that conferred resistance to lamivudine (14). These two reported patients were among the first to be described with this HBV mutation, of which the natural history has yet to be reported. In this report, we present the long term follow-up of these two previously reported patients.

PATIENTS AND METHODS

Both patients were previously reported as part of a lamivudine and liver transplantation phase II study (13). The first patient was also reported in a study by Tipples et al (14). Briefly, in accordance with the study protocol (13), both patients with end-stage liver disease secondary to chronic HBV and HBV DNA-positive serum were given lamivudine 100 mg/day orally four to five weeks before transplantation. At the time of transplantation, both patients were serum HBV DNA-negative (less than 5 pg/mL) by radiolabelled liquid hybridization assay (Abbott Genostics, Abbott Laboratories, Illinois). Postoperative lamivudine was administered via nasogastric tube at a dose of 300 mg/day, with dose reduction to 100 mg/day when oral administration was commenced. Immunosuppression induction consisted of cyclo-

sporine, azathioprine and tapering methylprednisolone. Maintenance immunosuppression consisted of cyclosporine, azathioprine and prednisone tapered over months. Serum HBV surface antigen (HBsAg), HBV surface antibody, HBV early antigen, HBV early antibody and HBV DNA were screened weekly during the initial two months, every two weeks for the next two months and then monthly until the occurrence of HBV allograft reinfection. After allograft reinfection, the frequency of serum HBV DNA testing was determined by clinical need.

Both patients were followed regularly and concurrently at UA and the University of British Columbia (UBC). Serum HBV DNA was measured by radiolabelled liquid hybridization assay (Abbott Genostics) at UA and by chemiluminescent molecular hybridization assay (Digene Hybrid Capture HBV DNA Assay, Maryland) at UBC. Due to the use of different hybridization test kits at the two universities, the measured serum HBV DNA values between centres were not comparable, although a reported conversion is as follows (15):

$$\text{Digene HBV DNA (pg/mL)} = (7 \times \text{Abbott HBV DNA} + 5) \text{ (pg/mL)}$$

The specific lamivudine-resistant HBV strain was isolated and identified in both patients at UA. The virus polymerase gene was amplified by polymerase chain reaction and the YMDD motif sequenced by methods previously reported (14).

CASE PRESENTATIONS

Patient 1: A former nursing assistant contracted HBV 10 years before transplantation as a result of a needle-stick injury, with subsequent development of end-stage cirrhosis and portal hypertension. She was 41 years of age at the time of liver transplantation and initially had an uncomplicated post-transplant course with unremarkable liver enzymes. An asymptomatic rise in liver enzymes eight months post-transplantation was investigated and found to be a result of allograft reinfection with HBV. Serum HBV DNA quantified at UA (Abbott) on post-transplant day (PTD) 240 was 465 pg/mL. Serum HBV DNA quantified at UBC (Digene) on PTD 251 was 3659 pg/mL, reflecting the difference in assays between the two centres. The strain of reinfecting HBV was subsequently analyzed (14) and found to contain a mutation in the YMDD motif conferring resistance to lamivudine. The dose of lamivudine was increased to 300 mg/day, with concurrent addition of famciclovir at a dose of 500 mg three times daily and intramuscular administration of HBIG (Abbott Laboratories). Serum HBV DNA (Abbott) declined to 164 pg/mL on PTD 263 with administration of this antiviral regimen.

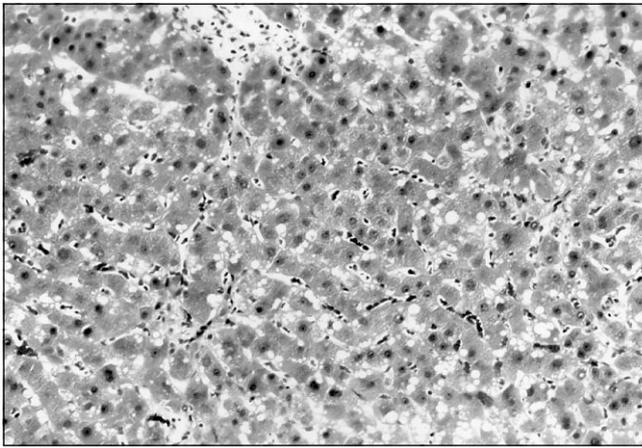


Figure 1) Liver biopsy from patient 1 taken 2.5 months after allograft reinfection with lamivudine-resistant hepatitis B virus revealing mild lobular hepatitis and normal architecture (hematoxylin and eosin, original magnification x40)

The liver was biopsied on PTD 314 as the result of a persistent mixed pattern of liver enzyme elevation (PTD 307: aspartate aminotransferase [AST] 61 U/L [normal less than 40 U/L], alanine aminotransferase [ALT] 57 U/L [normal less than 55 U/L], alkaline phosphatase 205 U/L [normal less than 125 U/L], gamma-glutamyltranspeptidase [GGT] 606 U/L [normal less than 50 U/L], total bilirubin 25 $\mu\text{mol/L}$ [normal less than 22 $\mu\text{mol/L}$). Biopsy revealed lobular hepatitis (Figure 1) and acute cellular rejection (mixed portal inflammatory infiltrate, biliary duct damage and endotheliitis). A trichrome stain did not reveal evidence of increased fibrosis. The acute rejection episode was treated with pulse methylprednisolone (500 mg intravenously daily for three doses).

Elevated liver enzymes subsequently returned to within normal limits by PTD 317. Repeat serum HBV DNA measurements at both centres revealed that levels had increased to 200 to 215 pg/mL (Abbott) and 6137 pg/mL (Digene) (PTD 383). Famciclovir was discontinued from the regimen, and HBIG was administered on a regular basis from PTD 376 to 445. HBsAg remained positive throughout the period of HBIG administration and anti-HBsAg titres were consistently undetectable. Further HBIG administration was discontinued due to lack of efficacy.

The patient's liver enzymes remained consistently within normal limits despite continuation of lamivudine as the sole antiviral agent. Despite unremarkable biochemistry, serum HBV DNA remained elevated, with a level greater than 2000 pg/mL (Digene) on PTD 527. Except for persistent right upper quadrant discomfort, the patient remained asymptomatic until 14 months after allograft reinfection with the lamivudine-resistant HBV strain. Development of abdominal distention was investigated with ultrasound, which revealed the presence of ascites (PTD 734). A transjugular liver biopsy with hepatic vein pressure measurements was performed on PTD 808. Hepatic vein wedged pressure (HVWP) was 26 mmHg, and hepatic vein free pres-

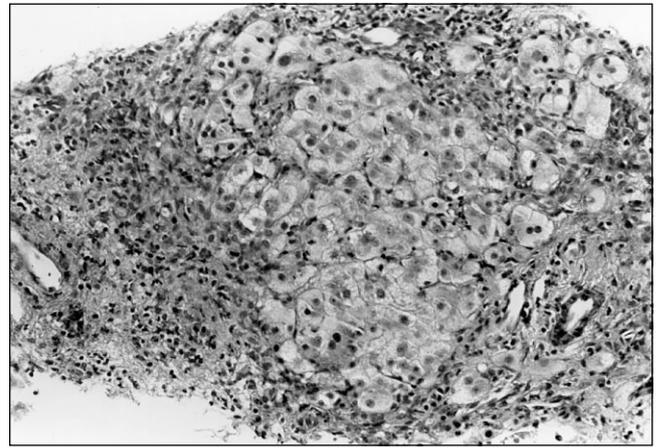


Figure 2) Liver biopsy from patient 1 taken 18 months after allograft reinfection showing cirrhosis with nodule formation and lobular hepatitis with piecemeal necrosis (periodic acid Schiff with distase, original magnification x40)

sure (HVFP) was 13 mmHg. The HVWP-HVFP gradient was 13 mmHg, which was consistent with portal hypertension (normal gradient less than 6 mmHg) (16). Liver biopsy revealed lobular hepatitis and cirrhosis (Figure 2). There was no evidence of acute or chronic rejection. The patient continues to be maintained on lamivudine.

Patient 2: The second patient was a man who was 55 years of age at the time of transplantation. He had a history of long standing chronic hepatitis, resulting in decompensated cirrhosis. His HBV infection was believed to be the result of vertical transmission. The post-transplant course was unremarkable until PTD 409, when his liver enzymes suddenly increased (peak enzymes PTD 429: AST 275 U/L, ALT 609 U/L, alkaline phosphatase 85 U/L, GGT 80 U/L, total bilirubin 190 $\mu\text{mol/L}$). The flare in serum enzymes was accompanied by symptoms of general malaise, nausea and vomiting. A liver biopsy on PTD 415 revealed minimal portal inflammation and moderate focal lobular hepatitis (Figure 3). There was no fibrosis or evidence of rejection. Immediately before the flare in liver enzymes, HBsAg reverted to seropositivity (PTD 393), having been repeatedly negative. Serum HBV DNA (Digene) on PTD 439 was 759.9 pg/mL. The re-infecting virus was subsequently sequenced (at UA) and found to contain a mutation in the YMDD motif.

The dose of lamivudine was increased to 300 mg/day; famciclovir 500 mg three times daily and weekly HBIG injections were added to the antiviral regimen. Symptoms of acute hepatitis resolved after two months. Liver enzymes returned to within normal limits by PTD 481. The enzymes, however, again became elevated to varying degrees shortly thereafter and have remained persistently elevated (PTD 846: ALT 84 U/L, AST 87 U/L, alkaline phosphatase 134 U/L, total bilirubin 15 $\mu\text{mol/L}$). Follow-up serum HBV DNA determinations (Digene) on PTD 457 and PTD 556 were 274.9 pg/mL and 1628 pg/mL, respectively. HBIG was discontinued three months after initiation due to lack of efficacy. Famciclovir was likewise discontinued after six months.

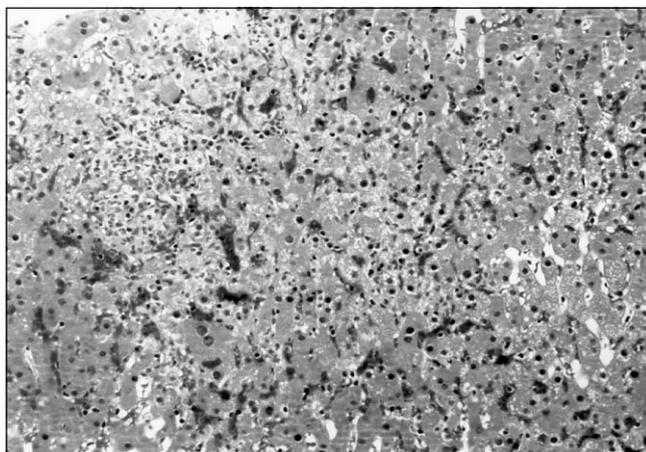


Figure 3 Liver biopsy from patient 2 revealing lobular hepatitis (hematoxylin and eosin, original magnification x40)

Sixteen months after allograft reinfection the patient is asymptomatic without clinical evidence of decompensated liver disease and remains on lamivudine.

DISCUSSION

Although HBV is a DNA virus, its replicative cycle requires RNA-dependent reverse transcription (17) similar to that of retroviruses such as HIV. Lamivudine, an antiretroviral agent, has been demonstrated to be effective in suppressing HBV replication in the nontransplant setting (10-12). The possibility of administering prophylactic lamivudine to liver allograft recipients transplanted because of chronic HBV infection has recently been studied. Aside from the present study (13), which reported only a 50% recurrence-free success rate in long term survivors, a 90% success rate of patients surviving the immediate postoperative period (n=10) has also been reported (18). The emergence of lamivudine-resistant strains of HBV was first reported simultaneously by us (14) and investigators in the United Kingdom (19). Since then, other transplant centres have, likewise, reported the emergence of lamivudine-resistance mutations (20,21). The emergence of lamivudine resistance has also been reported in the nontransplant setting (22). Although the incidence of emergence of these strains is not known, based on the patients reported in the literature the rate of development of resistant mutations is estimated to be on the order of 25% (13,18,21). This estimate, however, is based on very small numbers of study participants and short periods of observation.

To date, the lamivudine-resistant strains of HBV all contain a point mutation in a specific locus of the viral polymerase – the YMDD (tyrosine, methionine, aspartate, aspartate) motif, with isoleucine or valine substituting for methionine (14,19-22). This mutation in the YMDD region is identical to that identified in lamivudine-resistant strains of HIV (23). Because the YMDD mutation in HBV has only recently been reported, the long term natural history has, to date, not been described. Because no occurrences of fibrosing cholestatic hepatitis, an aggressive and almost al-

ways fatal form of allograft reinfection (24), have been reported, some have speculated that the YMDD mutation is less virulent than the wild type of HBV. Our experience suggests that lamivudine escape mutations may, in fact, be quite aggressive. Our first patient developed end-stage cirrhosis with both documented (increased HVWP-HVFP gradient) and clinically apparent portal hypertension (ascites) within 18 months of allograft reinfection with this strain. This patient did not suffer from chronic rejection, and her decompensated cirrhosis can only be attributed to the lamivudine-resistant strain of HBV. Her post-transplant course is similar to that experienced in the pre-lamivudine/HBIG era, with cirrhosis described within a year of transplantation (25).

The clinical course of our second patient has been less severe than that of the first patient. Although the patient was acutely symptomatic during the acute phase of allograft reinfection, these symptoms resolved as the flare of serum aminotransferases subsided. Sixteen months after allograft reinfection with the lamivudine-resistant strain of HBV, he is clinically well without evidence of portal hypertension. It is tempting to speculate that his long term clinical outlook will be favourable and analagous to that of patients with allograft reinfection with hepatitis C – chronic hepatitis with acceptable long term graft and patient survival (26). It must be noted, however, that this patient continues to have persistently elevated liver enzymes, and his follow-up period is still relatively short. It is too premature to state that the YMDD mutation, in the long term, is less virulent and that this patient's clinical course will continue to remain benign.

An additional point of interest is that both of these patients underwent a therapeutic trial of famciclovir for a significant period of time after the emergence of the lamivudine-resistant strain. Famciclovir is a nucleoside antiviral agent that has demonstrated efficacy in the treatment of herpetic infections (27). Famciclovir is the prodrug of the active compound penciclovir. Famciclovir or penciclovir has been demonstrated to suppress HBV replication in vitro (28) in a Pekin duck model (29) and in a small clinical trial (90% reduction of serum HBV in six of 11 patients) (30). Famciclovir therapy has also been reported to be of some success in the treatment of post-transplant allograft reinfection with wild-type HBV (31,32). In both of our patients, the addition of famciclovir did not appear to produce any significantly sustained reduction in serum HBV DNA levels. Although our experience with famciclovir and infection with lamivudine-resistant strains of HBV is limited to only two patients, it suggests that the YMDD mutation is not responsive to famciclovir therapy.

CONCLUSIONS

Our clinical experience with the YMDD-mutated strain of lamivudine-resistant HBV suggests that the strain can be aggressive. The resulting chronic hepatitis may be associated with significant morbidity and result in end-stage cirrhosis within a short period of time. To date, the available antiviral agents have been ineffective in suppressing this mutant strain.

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