

Lamivudine prophylaxis of liver allograft HBV reinfection in HBV related cirrhotic patients after liver transplantation

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BACKGROUND: Liver allograft hepatitis B virus (HBV) reinfection and hepatitis B (HB) recurrence jeopardize the long-term survival of recipient and liver allograft. Lamivudine has been referred as a novel antiviral agent against HBV in HBV cirrhotic patients even in liver transplantation setting. We assessed the prophylactic effect of lamivudine on liver allograft HBV reinfection and clarified the dynamic changes of HBV markers in HBV related decompensated liver cirrhosis after liver transplantation.

METHODS: Twenty-five recipients were divided into three groups: HBV active replication group (15 recipients), HBV inactive replication group (7), and control group (3). 100 mg/d lamivudine was administered preoperatively except in the control group. The HBV markers of serial sera and liver biopsy samples of the 25 recipients were evaluated regularly with enzyme-linked radioimmunoassay, HBV DNA fluorescent quantitative assay, immunohistochemical staining, labelled streptavidin biotin (LSAB) and digoxin labelled HBV DNA hybridization in situ. The dynamic alteration of HBV markers under lamivudine prophylaxis was observed.

RESULTS: In the HBV active replication group who had received lamivudine 2 weeks before liver transplantation, serum HBV DNA positive converted to negative by 80%. HBsAg of all recipients disappeared after liver transplantation, but corresponding antibodies of HBV appeared within one week after the operation. HBsAb 9/15, HBcAb 13/15

and HBeAb 11/15 appeared and subsided gradually within 24 weeks. HBV DNA in sera was kept negative; HBsAg, HBcAg and HBV DNA hybridization in situ of liver biopsy samples remained negative after use of lamivudine. Ten of the 15 recipients showed clearance of HBV, and per se HBV markers were undetectable both in serum and liver biopsy samples between 12 to 44 weeks (24 weeks on average). The 1-, 2-year survival rates were 83% in this group. Two of the 15 recipients developed HBV allograft reinfection or recurrence of hepatitis 2 years after lamivudine monoprophyllaxis (2/15, 13.3%). In the HBV inactive replication group, the outcome was similar to that of the HBV active group. The HBV antibody frequency was HBsAb 4/7, HBcAb 6/7, and HBeAb 2/7. Three of 7 recipients showed HBV clearance both in sera and liver biopsy samples, whereas in the control group all 3 recipients developed HBV allograft reinfection and recurrent hepatitis 8, 10, 12 months postoperatively; one of them died of fibrosing cholestatic hepatitis, and the remaining 2 recovered after additional lamivudine therapy. The overall allograft reinfection rate was 9.1% (2/22) and the overall 1-, 2-year survival rates were 87% in the lamivudine prophylaxis group.

CONCLUSIONS: Lamivudine prophylaxis can prevent effectively liver allograft from HBV reinfection in patients with HBV-related decompensated liver cirrhosis even in HBV active replication recipient after liver transplantation. Its long-term outcome remains to be studied.

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KEY WORDS: liver cirrhosis; hepatitis B virus; liver transplantation; lamivudine

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Introduction

Although liver transplantation (LT) is the only promising method to cure HBV-related end-stage hepatopathy, it has promised not too much up to now because the reinfection rate is as high as 70%-80%, with a 1-year survival rate of 68% and a 3-year survival rate of 44%.^[1] Since the late 1990s, liver transplantation has been expanding throughout China with

more than 500 new recipients each year, of whom over 80% are HBV-related and over 50% have active HBV replication.^[2] This kind of risk is becoming more and more important while recipients and allografts have a longer survival period. It has been reported that the reinfection of liver allograft after liver transplantation could be prevented and treated successfully through the administration of lamivudine plus hepatitis B immunoglobulin (HBIG),^[3-9] but similar studies are not reported in China. From February 1999 to December 2001, we systematically used lamivudine on 25 out of 50 liver transplantation patients to prevent the reinfection of HBV and conducted a non-randomized control study with a follow-up of 6-36 months.

Methods

Patients

In 50 recipients who had LT from February 1999 to December 2001, 41 (82%) were due to end-stage hepatopathy with HBsAg (hepatitis B surface antigen) positive. 22 (54%) of these patients had active HBV DNA replication or/and positive HBeAg, but 7 of them died soon after surgery because of causes irrelevant to HBV reinfection, and they were excluded. Other 19 patients had only positive HBsAg or positive HBV markers on their native livers detected by immunohistochemistry with inactive HBV DNA replication. Of the 19 patients, 8 had primary liver cancer and 1 died soon after surgery from pulmonary infection, so 10 patients were also excluded. At last a total of 25 patients in our study were followed up for 6-36 months (Table 1). The recipients included were those with preoperative decompensated hepatic disease showing HBsAg positive in serum or/and HBV DNA active or/and HBcAg immunohistochemically positive in their liver tissue. Those with anti-HCV positive or cytomegalovirus IgG/IgM positive or HBcAb positive were excluded from this study. Those who needed long time anti-virus therapy were not included either because the activeness of HBV was

disturbed. Informed consent was obtained from all patients in this study and the study was approved by the ethics committee of the hospital.

Methods

The 25 patients were divided non-randomized into two groups of active HBV DNA replication group (15 patients with HBV DNA positive or/and HBeAg positive) and inactive HBV DNA replication group (10 patients with HBsAg positive but HBV DNA/HBeAg negative). Of the latter group, 3 patients were not given lamivudine because of its unavailability in China before 2000, and they acted unintentionally as negative controls. The administration of lamivudine was as follows: 100 mg po qd for as early as possible before operation and 100 mg po qd in two years after operation. The dynamic alteration of HBV markers in liver tissue and serum was observed and the 1- and 2-year survival rates were documented. Successful prevention and treatment was defined as the following items:^[1,9-12] clinical clearance, immunohistochemically negative HBV markers in serum and liver tissue; negative HBV DNA in serum assessed by fluorescent quantitative method; negative HBV DNA in liver tissue determined by hybridization in situ; effective antibodies alone present in serum; HBV clearance and HBsAb rising up to 10 IU/L^[12] and lasting for more than 2 years spontaneously or after vaccination. Prevention and treatment failed when HBsAg positive in serum, or HBV DNA positive revealed by fluorescent quantitative method, or HBV markers positive in liver tissue could be considered HBV reinfection. These indexes plus elevated levels of bilirubin or transaminase and clinical manifestations could be considered HB recurrence. HBeAg negative and HBV DNA positive could be explained as failure of lamivudine prophylaxis.

Enzyme-linked immunoassay method (Abbott Laboratories, North Chicago, IL, USA) was used to detect HBsAg, HBeAg and corresponding antibodies. HBV DNA fluorescent quantitative analysis was made by the Hepatitis Research Lab of this hospital, with a lower value of 0.05 pg/ml (10³ copy/ml of HBV DNA by PCR). HBsAg and HBcAg in liver tissue were detected by SLAB method (from BioGenex, San Romon, CA 94583, USA). HBV DNA in liver tissue was measured by hybridization in situ method labelled by digoxin (probe label box bought from Behringer Mannheim GmbH, Germany), and digoxin antibody purchased from Roche Diagnostics Corporation, Indianapolis, USA. Biopsy specimens were taken regularly from the native host liver, the allograft before implantation, the transplanted allograft before closing the abdomen, and 1, 4, 12, 24, 48, 72, 96 weeks or whenever biopsy was needed respectively after transplantation. Informed consent was obtained from the patients and their families for each needle biopsy, and the coupled samples were judged by

Table 1. The data of the 25 patients at the beginning of the study

Age (y)	39(25-58)
Male/female	22(88%)/3(12%)
Average time after LT (mon)	12(6-36)
Serum HBsAg positive (%)	25(100)
HBeAg positive (%)	13(52)
Immunohistochemical staining HBsAg(+)	20(80%)
HBcAg(+)	10(40%)
HBV DNA hybridization in situ positive	15(75%)
HBV DNA fluorescent quantitatively positive	9.2×10 ⁹ copy/L
ALT	60 IU/L
Bilirubin level in serum	120 μmol/L
Albumin level in serum	35(25-47) g/L

two pathologists who knew nothing about the patients and their clinical data. The criteria for judgement included the degree of liver lesions and the staining of HBsAg and HBcAg by peroxidase.

Statistical analysis

All data were represented as means \pm standard deviation; the long-term survival rate was analyzed using the Kaplan-Meier method.

Results

Dynamic alteration of HBV markers in recipients with active HBV replication

The average HBV DNA was 9.2×10^6 copy/ml in the 15 patients with active HBV replication at the beginning. After being given lamivudine for 2 weeks before operation, 12 of them (80%) showed HBV DNA in their serum. Only those patients who had had preoperative lamivudine administration less than 1 week were HBV DNA positive shown by fluorescent quantitative method. All the patients in this group continued using lamivudine after surgery. HBsAg and HBeAg remained negative in the serum, and the corresponding antibodies appeared as early as 1 week after operation. Nine patients had HBsAb, 13 had HBcAb and 11 had HBeAb; all these antibodies disappeared gradually within 6 months. In patients who were HBsAb positive 1 week after operation, the average amount of antibodies was more than 164 IU/L, which was considered to be related to the blood and blood products administered. The HBV DNA quantity in these patients remained undetectable using fluorescent quantitative method. The bilirubin and ALT levels in serum increased moderately in 2 weeks, decreased after 3 weeks, and normalized 3 months after

operation. Needle biopsy of the liver 1-4 weeks after surgery found that only 2 patients had mild positive reaction of HBsAg, 1 patient was HBcAg positive, and all HBV markers described as above became negative in 3 months. All patients remained HBV DNA negative after digoxin-marked HBV DNA hybridization in situ (Table 2). Follow-up for 12-44 weeks showed that HBV markers disappeared in serum and liver tissue in 10 patients, with an average disappearance time of 23.7 ± 13.05 weeks and 4 weeks respectively. The clinical clearance was irrelevant to the HBV DNA level in serum before operation.

One patient with clinical clearance at 1 year showed HBsAb 764 IU/L in the first week postoperatively, 84.5 IU/L at the 24th week, and less than 2 IU/L at 1 year. At the end of 2 years, HBsAg became positive, HBV DNA fluorescent-quantitatively positive and HBeAg negative with no corresponding elevation of bilirubin and transaminase. This phenomenon could actually be YMDD variation (1/22, 4.6%). Unfortunately, another patient died from HB recurrence at 2 years; at 1 year the patient showed good outcome of clinical clearance in this group.

Dynamic alteration of HBV markers in recipients with inactive HBV replication

The results of using lamivudine in 7 recipients with inactive HBV replication were similar to those in patients with active HBV replication. HBsAg disappeared immediately after operation, and the corresponding antibodies could be detected within 1 week postoperatively (HBsAb 4/7, HBcAb 6/7, HBeAb 2/7). These antibodies lasted for about 24 weeks, but HBcAb and HBeAb lasted longer than HBsAb. The HBV markers disappeared in 1-2 years in 3 patients (Table 3). HBV remained immunohistochemically negative in liver tissues for 6 mon-

Table 2. Dynamic alteration of HBV markers in serum and liver tissue in recipients with active HBV replication under lamivudine prophylaxis (15 patients)

HBV marker	Preoperation	After operation	Weeks									
			1-	2-	4-	8-	12-	24-	48-	60-	72-	96-
Marker in serum												
HBsAg	15/15		1/15	0/15	0/15	0/15	0/15	0/13	0/5	0/4	0/4	1/2
HBeAg	6/15		0/15	0/15	0/15	0/15	0/13	0/5	0/4	0/4	0/2	
Anti-HBs	0/15		9/15	10/15	5/15	2/15	1/13	0/5	0/4	0/4	0/2	
Anti-HBc	15/15		13/15	15/15	9/15	5/15	3/13	0/5	0/4	0/4	1/2	
Anti-HBe	3/15		11/15	10/15	7/15	3/15	1/13	0/5	0/4	0/4	1/2	
HBV DNA	9.2×10^9		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	0/2
Bilirubin level ($\mu\text{mol/L}$)	37.71 ± 10		157.15 ± 30	70 ± 9	50 ± 8	38 ± 6	<28	<28	<28	<28	<28	<28
ALT (IU/L)	40 ± 10		150 ± 50	100 ± 10	60 ± 20	50 ± 20	30 ± 10	30 ± 10	30 ± 10	30 ± 10	30 ± 10	
Marker in liver tissue												
HBsAg	10/15	0/15	2/15		2/15		0/15	0/13	0/5	0/4	0/4	0/2
HBcAg	10/15	0/15	0/15		1/15		0/15	0/13	0/5	0/4	0/4	0/2
HBV DNA hybridization in situ	10/15	0/15	0/15		0/15		0/15	0/13	0/5	0/4	0/4	0/2

Table 3. Dynamic alteration of HBV markers in serum and liver tissue in recipients with inactive HBV replication under lamivudine prophylaxis (7 patients)

HBV marker	Preoperation	After operation	Weeks									
			1-	2-	4-	8-	12-	24-	48-	96-	144-	
Marker in serum												
HBsAg	5/7			0/7	0/7	0/7	0/7	0/7	0/7	1/6	0/4	0/1
HBeAg	0/7			0/7	0/7	0/7	0/7	0/7	0/7	0/6	0/4	0/1
Anti-HBs	0/7			0/7	4/7	4/7	4/7	3/7	4/7	1/6	2/4	1/1
Anti-HBc	7/7			4/7	6/7	6/7	6/7	5/7	5/7	2/6	0/4	0/1
Anti-HBe	2/7			6/7	2/7	2/7	2/7	2/7	2/7	2/6	0/4	0/1
HBV DNA by PCR	Negative			Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative
Bilirubin level in serum	Mildly elevated			Negative	Almost normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
ALT	Mildly elevated			Elevated	Almost normal	Normal	Normal	Normal	Normal	Normal	Normal	Normal
HBV marker in liver tissue												
HBsAg	7/7	0/7	0/7		0/7	0/7	0/7	0/7	2/7	2/7	0/4	0/1
HBeAg	7/7	0/7	0/7		0/7	0/7	0/7	0/7	2/7	1/7	0/4	0/1
HBV DNA hybridization in situ	0/7	Negative	Negative		Negative	Negative	Negative	Negative	Negative	Negative	Negative	Negative

Table 4. Dynamic alteration of HBV markers in serum and liver tissues in recipients with inactive HBV replication without use of lamivudine (3 patients)

HBV marker	Preoperation	After operation	Weeks									
			1-	2-	4-	8-	12-	24-	48-	96-	144-	
Marker in serum												
HBsAg	3/3			0/3	0/3	0/3	0/3	0/3	3/3	3/3	1/2	0/1
HBeAg	0/3			0/3	0/3	0/3	0/3	0/3	0/3	1/3	0/2	0/1
Anti-HBs	0/3			3/3	3/3	3/3	3/3	0/3	0/3	0/3	0/2	0/1
Anti-HBc	3/3			3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/2	1/1
Anti-HBe	2/3			3/3	3/3	3/3	3/3	3/3	3/3	3/3	2/2	1/1
HBV DNA by PCR	Negative			Negative	Negative	Negative	Negative	Negative	Negative	5.6×10 ⁸	Negative	Negative
Bilirubin	Mildly elevated			Elevated	Elevated	Elevated	Elevated	Elevated	Almost normal	Elevated	Normal	Normal
ALT	Mildly elevated			Elevated	Elevated	Elevated	Elevated	Elevated	Normal	Mildly elevated	Mildly elevated	Mildly elevated
Marker in liver tissue												
HBsAg	3/3	0/3	0/3		0/3			1/3	3/3	2/3	1/2	0/1
HBeAg	3/3	0/3	0/3		0/3			0/3	3/3	3/3	1/2	0/1
HBV DNA hybridization in situ	Negative	Negative	Negative		Negative			Negative	Negative	Positive	Negative	Negative

ths. Although HBeAg and HBsAg became positive in liver tissues in two patients after half a year, no change was observed in biochemical indexes and HBV DNA remained negative after hybridization in situ (Table 3).

Dynamic alteration of HBV markers in 3 controls

In the 3 patients who were not given lamivudine, their HBsAg and HBeAg disappeared immediately after operation and the antibodies emerged within 1 week and existed for half a year. HBsAg in liver tissues was positive at the 12th week, and HBsAg and HBeAg were detected in half a year. Eight–12 months after operation, HBV DNA in serum became positive and the levels of

bilirubin and transaminase elevated. HBV DNA was positive after hybridization in situ at the same time. In 1 patient dead liver tissue showed the characteristics of fibrosing–cholestatic hepatitis. In 2 patients who were treated comprehensively including the use of lamivudine, HBV DNA became negative with decreased levels of bilirubin and transaminase (Table 4). Follow-up revealed that 1 patient in the control group died from HB recurrence (fibrosing–cholestatic hepatitis), from the cerebral haemorrhage irrelevant to HB recurrence, and still 1 from HB recurrence because of allograft HBcAb positive. According to the mentioned definition, the 24–144-week clinical clearance of HBV in the 22 patients in

the two groups was at least 59% (13/22), HBV reinfection was 9% (2/22), HB recurrence and YMDD variation were 4.5% (1/22) respectively, and one patient died from HB recurrence. The clinical clearance in the control group was 0 (0/3), HBV allograft reinfection and HB recurrence were 100% (3/3); one (33%) died in the first year and the death rate was 33% (1/3).

Discussion

In the 1990s, Samuel et al^[1] reported that HB recurrence rate in patients with active HBV replication after LT was 90%-100%. HBIG was once used to prevent such recurrence reducing the rate to 33%, but still not satisfactory for HBV DNA positive patients.^[1,9] Hence patients with active HBV replication were strongly contraindicated for LT. Greillier in 1996 reported 10 LT patients with HBV DNA positive who were given lamivudine. In the following 18-90 weeks, HBsAg in 9 patients became negative at the 24th week, HBV DNA in 1 patient remained positive and recurred at the 72nd week. From that time on, lamivudine has been used widely to prevent HB recurrence in LT recipients for HB related hepatopathy, and this kind of disease is indicated again for LT.^[13-18] Because of many HB patients in China and other Asian countries the improvement of this remedy is of special significance.^[19]

In our study, 80% (12/15) recipients with HBV DNA positive showed decreased HBV DNA in serum to an undetectable level when lamivudine was given 2 weeks before surgery. Administration of lamivudine less than 7 days could hardly make HBV DNA negative. This effect is seemingly better than that in HBV active replicating patients receiving no liver transplantation.^[20] Using lamivudine decreased HBV particles in blood circulation before LT as many as 97%, thus decreased the chance for virus to reenter the allograft. Our study also confirmed the advice that the recipients with active HBV replication should use lamivudine at least for 2 weeks ahead of surgery to prevent HB recurrence.^[2] The markers in serum in patients who had received lamivudine prophylaxis regime dropped sharply, HBsAg and HBeAg disappeared after surgery, and the corresponding antibodies emerged within 1 week (HBsAb 9/15, HBeAb 11/15, HBcAb 13/15) and existed for 24 weeks. Most importantly, HBsAb showed an emergence rate of over 60%. In another study we suggested that it could possibly be introduced during transfusion of blood and blood products after operation.^[21,22] In a week, its concentration in serum could be higher than 160 IU/L, recognized as an effective protective level.^[1,13] Subsequently, HBsAb began to decrease significantly within 6 months. Needle liver biopsy and HBV DNA hybridization in situ showed no reappearance of HBV markers, indicating that allograft is well protected. A small volume of HBs-

Ag and HBcAg was shown immunohistochemically in liver tissues in the 1st-4th weeks, but they soon became negative. If experimental error could be excluded, the following auto-clearance mechanisms of recipients might be involved; promptly or rapid decrease of HBV DNA particles and inhibition of HBV replication after lamivudine administration; elimination of the host nest of HBV in the native liver; dilution of viruses in serum after intraoperatively transfusion of a large volume of blood and fluid; HBsAb neutralization of HBV viruses in over 60% patients who receive HBsAb passively and unconsciously. The stage was called security period under lamivudine coverage (may conjoined with HBIG) after LT operation. HB recurrence was not observed in patients of this stage and HBV was cleared clinically in over 59% patients. In contrast, other reports showed that HB recurrence was within half a year.^[1,9]

Twelve-44 weeks after operation in this study, HBV markers in serum and liver tissues became negative in 10 of 15 (66.7%) recipients within an average of 24 weeks. Clinical clearance was obtained but no complete elimination of HBV was indicated. Because the replicating mechanism of HBV remained unclear and the observation time was short, more time was needed for further evaluation.

In our study one patient (4.5%) had YMDD variation after lamivudine administration for 2 years. We consider that despite the use of both lamivudine and HBIG, HB recurrence may indicate the risk of virus variation. In case of clinical clearance, it might be necessary to stimulate active immunity in recipients after HBV vaccination to protect the hosts from HBV infection.^[12]

It was reported that the 1-year recurrence rate in patients with HB related hepatopathy after LT was 70%-80%, and the actual 2-year survival rate was 54%.^[1] HBV could be activated again under immunosuppressive state in recipients with inactive replicative HB related hepatopathy (HBsAg+, HBV DNA-).^[14] Three patients in our control group were not given lamivudine promptly, resulting in HBV DNA positive at the 8th month, HBsAg and HBeAg in serum positive at the 10th month, and HBsAg and HBcAg in liver tissue positive at the 12th month. On the contrary, no one had HB recurrence in 7 recipients who received lamivudine, and 3 of them had clinical clearance. Further, no recurrence was observed in a year. This result showed that lamivudine prophylaxis is reasonable and necessary to prevent HBV allograft reinfection and HB recurrence even in patients with inactive HBV replication after LT.^[23-28]

In our study, 22 patients had one HB recurrence on average after follow-up for one year except for YMDD variation in one patient (4.5%), which was less than 14% reported by Lai with 1- and 2-year survival rates of more than 83%. In our control group, the rates

of HBV allograft reinfection and HB recurrence were 100%, and the mortality was more than 33%. This study has demonstrated that lamivudine is used satisfactorily to prevent HBV allograft reinfection and HB recurrence after LT, but the clinical significance of HBsAb emergence demands further study.^[7]

Competing interest

No benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article.

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In science, read, by preference, the newest works; in literature, the oldest.
The classic literature is always modern.

— Edward Bulwer Lytton