Impact of human leukocyte antigen matching on hepatitis B virus recurrence after liver transplantation

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BACKGROUND: **Liver transplantation (LT) is an effective therapy for end-stage hepatitis B virus (HBV) infection. Recurrence of HBV is one of the frequent complications. In the present study, we investigated whether human leukocyte antigen (HLA) matching influences the incidence of HBV recurrence, and the time point of HBV recurrence after LT.**

METHODS: **One hundred and two recipients of LT with end-stage chronic HBV infection were reviewed. The triple-drug immunosuppression regimen consisted of tacrolimus, mycophenolate, and prednisone. All patients were subjected to prophylaxis with hepatitis B immunoglobulin and lamivudine. HLA typing was performed using a sequence-specific primer-polymerase chain reaction kit. Serology for hepatitis B and HBV DNA was examined using a commercial kit.**

RESULTS: **The incidence of recurrent HBV infection post-LT was 6.86%. The recurrent infection of HBV was independent of the degree of HLA matching (P>0.05). The time point of HBV recurrence, however, was prolonged in HLA-A matched patients compared with matchless patients (P=0.049). The recurrence of HBV infection was independent of HLA compatibility.**

CONCLUSIONS: **This retrospective analysis showed that more HLA-A locus compatibility is associated with a prolonged time of recurrence of HBV in patients after LT for end-stage HBV infection. The incidence of HBV recurrence is independent of HLA compatibility.**

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KEY WORDS: **liver transplantation; human leukocyte antigen; hepatitis B virus; recurrence**

Introduction

Liver transplantation (LT) is an effective therapy for end-stage chronic liver disease.[1-3] The organs used for LT are usually selected according to blood type (ABO) and matched for body size with the transplant recipient. In kidney or bone marrow transplantation, the degree of human leukocyte antigen (HLA) matching between donor and recipient has a significant clinical impact.[4, 5] The value of HLA matching in LT, however, is controversial, with improved outcomes in some cases, poor outcomes in others, and no effect in the remaining cases.[6, 7] Hepatitis B virus (HBV) can lead to intracellular infection, while MHC-1 may play an important role in this process. MHC class Ⅰ-restricted $CD8^+$ T cells can recognize HBV peptides, which lead to direct cell killing by $CD8^+$ cytotoxic lymphocytes.[8, 9] A number of plasma HBV antigens may be processed by macrophages and presented to $CD4^+$ cells by a similar mechanism.[10] Thus, the match of HLA-Ⅰ and -Ⅱ molecules between donor and recipient may affect the recurrence of HBV.[11, 12] In this study, we analyzed whether HLA matching influences the incidence of HBV recurrence and the time point of HBV recurrence after LT.

Methods

The study was conducted in our single center. Some patients were excluded for the following reasons: death within one month after transplantation, ABO blood type incompatibility, missed follow-up visit, co-infection with other hepatitis virus, or imperfect HLA-typing. A total of 102 patients with end-stage chronic HBV infection in our hospital were included in the analysis.

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There were 87 males and 15 females, whose ages ranged from 15 to 68 (mean 45.55±10.23 years). The donors were 69 living and 33 cadaveric individuals. Modified LT was performed in recipients.[13] The selection criteria of donors and recipients were based on their age, blood type, graft size, and liver function. Every organ donation and transplantation in our center strictly followed the guidelines of the Ethics Committee of our hospital and the *Declaration of Helsinki*.

All patients were subjected to prophylaxis with intramuscular hepatitis B immunoglobulin (HBIg) and oral lamivudine. Lamivudine (100 mg/d) was administered post-LT. The dosage of HBIg was on an unfixed schedule consisting of 2000 IU in the anhepatic phase, followed by 800 IU daily for the next 6 days and weekly for 3 weeks, and then 800 IU monthly thereafter.[2]

The immunosuppressive regimen consisted of a triple-drug regimen with tacrolimus, mycophenolate, and prednisone. The dose of tacrolimus was adjusted to maintain a level of 7-10 ng/ml for the first postoperative month and 5-7 ng/ml thereafter. The dose of mycophenolate was 500 mg bid by oral administration. Prednisone was discontinued after 3-6 months. Follow-up with regular virological and biochemical monitoring was conducted weekly on an outpatient basis, and then monthly after the condition became stable. Patients were examined by hepatitis serology and HBV DNA at regular intervals in our outpatient clinic. Recurrent HBV infection of the liver graft was diagnosed by detection of HBsAg and HBV DNA in recipient sera.[10] Graft failure was confirmed histologically by graft biopsy.

HLA typing of the recipients and donors was detected by a sequence-specific primer-polymerase chain reaction kit (One Lambda Inc., Canoga Park, CA, USA) according to the manufacturer's instructions as previously described.[14] HBsAg, HBeAg, anti-HBs, anti-HBe, and anti-HBc were monitored regularly using commercially available test kits (PiJi Co., Shenzhen, China). HBV DNA was detected by PCR using a commercial kit (PiJi Co., Shenzhen, China) with an approximate limit of detection of 1000 copies/ml.

All statistical analyses were performed using SPSS 16.0 (Chicago, IL, USA). HBV DNA levels and the time point of HBV recurrence are given as median and range. The Chi-square test was performed for dichotomous variables. The K independent-samples test was used to compare differences in the medians of continuous variables. Multivariate analysis of risk factors for HBV recurrence was made with binary logistic regression analysis. A P value of less than 0.05 was considered statistically significant.

Results

HBV recurrence

After LT, the patients were followed for a median of 12 months. HBV recurrence after LT was noted in 7 (6.86%) patients, with a median recurrence time of 12 months, and a median HBV DNA copy number of 6.7×10^6 copies/ml. Only one case of graft failure with HBV recurrence was found.

In the multivariate analysis, the following factors were not statistically correlated with the incidence of HBV recurrence: sex, age, donor, HLA-A, -B, or -DR compatibility between donor and recipient.

Relationship between donor/recipient HLA compatibility and outcome

The median preoperative HBV DNA level was 3.4×10^5 copies/ml in patients with no HLA-A compatibility, and 2.3×10^5 copies/ml in patients with one HLA-A compatibility ($\chi^2=0.697$, $P=0.706$). The HBV DNA and HBsAg tested positive in 7.9% (5/63) of the patients with HLA-A mismatch, 5.6% (2/36) with one HLA-A compatibility, and zero with mismatch ($\chi^2=0.427$, $P=0.808$; Table). The HBV DNA levels were similar among these groups ($\chi^2=3.750$, $P=0.053$; Table). The time point of HBV recurrence was significantly prolonged in patients with one or two HLA-A compatibilities compared with HLA-A mismatch (24 vs. 6 months, $\chi^2=3.889$, $P=0.049$; Table). Only one patient in the one HLA-A compatible group had graft failure with HBV recurrence.

The median preoperative HBV DNA level was 3.4×10^5 copies/ml in patients with no HLA-B compatibility, and 5.3×10^5 copies/ml in patients with one HLA-B compatibility ($\chi^2=0.410$, $P=0.815$). HBV DNA and HBsAg were tested positive in 8.2% (5/61) of patients without HLA-B compatibility, in 6.7% (2/30) of patients with one HLA-B match, and zero in patients with two compatibilities. And the incidence of HBV recurrence was not significantly different in patients with one or two HLA-B compatibilities ($\chi^2=0.973$, $P=0.615$). The HBV DNA levels were similar among the three groups ($\chi^2=0.600$, $P=0.439$; Table). The time point of HBV recurrence in patients with one HLA-B locus compatibility was similar to that in patients with two compatibilities (12 vs. 16.5 months; $\chi^2=0.039$, $P=0.844$; Table). Only one case of graft failure with HBV recurrence occurred in the no HLA-B compatible group.

The median preoperative HBV DNA level was 3.1×10^5 copies/ml in patients with no HLA-A or -B compatibility, 6.2×10^5 copies/ml in patients with one HLA-A or -B compatibility, and 4.0×10^5 copies/ml in

Table. Incidence of HBV infection and the time point of HBV recurrence with HLA matching

HLA-matching	HBV recurrence (n=7)	Copies of HBV DNA (copies/ml)	Time point of HBV recurrence (months)
A-locus			
0 (n=63)	5 (7.9%)	3.9×10^7 (1.5×10^6-2.2×10^9)	6 (3-12)
1 (n=36)	2 (5.6%)	4.9×10^4 (2.0×10^4- 8.0×10^4)	24 (18-30)
2 (n=3)	0	0	0*
χ^2 test	0.427	3.750	3.889
P value	0.808	0.053	0.049
B-locus			
0 (n=61)	5 (8.2%)	6.7×10^6 (8.0×10^4-2.2×10^9)	12 (3-18)
1 (n=30)	2 (6.7%)	2.0×10^7 (2.0×10^4-3.9×10^7)	16.5 (3-30)
2 (n=11)	0	0	0
χ^2 test	0.973	0.600	0.039
P value	0.615	0.439	0.844
HLA-A or B locus			
0 (n=45)	4 (8.9%)	1.1×10^8 (1.5×10^5-2.2×10^9)	9 (3-12)
1 (n=34)	2 (5.9%)	2.0×10^7 (8.0×10^4-3.9×10^7)	10.5 (3-18)
2 (n=90)	1 (11.1%)	1.5×10^4	30
3 (n=14)	0	0	0
χ^2 test	1.160	2.893	2.735
P value	0.657	0.235	0.305
DR-locus			
0 (n=59)	4 (6.8%)	3.2×10^6 (8.0×10^4-2.2×10^9)	9 (3-18)
1 (n=42)	3 (6.0%)	3.9×10^6 (2.0×10^4-2.2×10^8)	12 (3-30)
2 (n=1)	0	0	0
χ^2 test	0.097	0.000	0.130
P value	0.961	1.000	0.719

*: P<0.05.

patients with two HLA-A or -B compatibilities (χ^2=0.469, P=0.926). The incidence of HBV recurrence was 8.9% (4/45) in those who shared four HLA-A and -B antigens with their donors. The risk of HBV recurrence was 5.9% (2/34) for one HLA locus match and 11.1% (1/11) for two HLA locus matches (χ^2=1.160, P=0.657; Table). The HBV DNA levels were similar among the four groups (χ^2=2.893, P=0.235; Table). In addition, the time point of HBV recurrence among patients some HLA locus match was not prolonged compared to patients with HLA incompatibility (χ^2=2.735, P=0.305).

The median preoperative HBV DNA level was 6.8×10^5 copies/ml in patients with no HLA-DR compatibility, and 1.0×10^5 copies/ml in patients with one HLA-DR compatibility (χ^2=0.410, P=0.815). No significant association was found in the incidence of HBV recurrence between donor and recipient HLA-DR sharing (χ^2=0.097, P=0.961; Table). The HBV DNA levels were similar in the different groups (χ^2=0.000, P=1.000; Table). The median time points of HBV recurrence were 9 and 12 months in patients with zero or one HLA-DR compatibility, respectively (χ^2=0.130, P=0.719; Table). One patient who experienced graft loss with recurrent HBV was observed in those without HLA-DR compatibility.

Discussion

Chronic HBV infection is common, particularly in China. According to the reports of the World Health Organization, about 2 billion people are infected with HBV globally and 350 million of them are infected chronically. LT is currently the accepted effective treatment for end-stage liver disease and fulminant hepatitis due to HBV infection.[15] In addition, HBV recurrence is a severe and frequent complication in patients undergoing LT.[10, 15, 16]

Possible factors which affect recurrent HBV infection following LT include the size of the initial HBV inoculum,[17] the dose of immune suppression,[18] the copy numbers of HBV DNA pre-transplantation,[19] HBsAg positivity prior to LT,[10, 19] anti-HBc positivity in the donor,[20-22] receiving an HBV-positive donor liver, and HLA matching between the allograft and recipient.[11, 23] In this study, none of the donors was

positive for HBV DNA or anti-HBc antibodies, and the HBV DNA copy number was not significantly different in each group pre-transplantation. Meanwhile, in the multivariate analysis, the following factors were not statistically correlated with the incidence of HBV recurrence: sex, age, donor, HLA-A, -B, or -DR compatibility between donor and recipient.

In this study, the time point of recurrent HBV infection was prolonged in patients with HLA-A compatibility. HBV can lead to intracellular infections, and MHC-1 may play an important role in this process, with MHC class Ⅰ-restricted $CD8^+$ T cells recognizing HBV peptides, which leads to direct cell killing mediated by $CD8^+$ cytotoxic lymphocytes.[8, 9, 11] A number of plasma HBV antigens may be presented by macrophages and presented to $CD4^+$ cells via a similar mechanism.[10, 12] HLA molecules present foreign HBV antigens to both $CD4^+$ T lymphocytes and $CD8^+$ cytolytic T cells, leading to humoral and cell-mediated immune responses aimed to eliminate HBV.[24] In our study, the time point of HBV recurrence was independent of HLA-B and -DR compatibility.

Meanwhile, the rate of recurrence was lower than the reported previously.[10] It has been suggested that prophylaxis with HBIg and lamivudine reduces the incidence of HBV recurrence after transplantation.[2, 3, 25] Only one case of graft failure with HBV recurrence occurred in our study.

The impact of HLA matching is controversial, with some reports suggesting no association between HLA locus match and HBV recurrence.[6, 7, 26] And further research into the impact of HLA matching on HBV recurrence is warranted.

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Ethical approval: Organ donation and transplantation in our center strictly followed the guidelines of the Ethics Committee of our hospital and the *Declaration of Helsinki*.

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Proverbs are short sentences drawn from long experience.

—Miguel de Cervantes