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Lamivudine therapy for hepatitis B in renal transplantation

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Abstract

Antiviral therapies are associated with an increased risk of acute rejection in transplant patients. The aim of the present study was to evaluate the efficacy and safety of lamivudine therapy for hepatitis B virus (HBV) infection in renal transplant patients. Six patients were included in this study. They received 150 mg/day of lamivudine during a follow-up period of 24 months. The laboratory tests monitored were HBV DNA, HBsAg, HBeAg, ALT, γ-GT, serum creatinine and blood cyclosporine levels. The HBV DNA became undetectable in four patients as early as in the third month of treatment. After six months, the viral load was also negative in the other two patients, and remained so until 18 months of follow-up. The medication was well tolerated with no major side effects. Lamivudine was safe and effective in blocking HBV replication in renal transplant patients without any apparent increase in the risk of graft failure for the 24-month period of study.

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Hepatitis B virus (HBV) infection in patients with end-stage renal disease undergoing hemodialysis or submitted to renal transplantation generally has an unfavorable course with a tendency towards chronicity and causing important mortality (1).

Despite the decrease in the incidence of HBV in patients with chronic renal failure due to preventive measures, such as vaccination and practices of isolation, this infection is still considered of epidemiological importance in dialysis and transplantation units in Brazil. The frequency of HBV seropositivity in Brazil varies from 7.6 to 12% in hemodialysis patients (2).

It is still controversial whether a patient with chronic HBV infection should be maintained on dialysis or submitted to transplantation (1,3). The real impact of transplantation and treatment with immunosuppressive drugs on patients with chronic hepatitis B is not clear. It is known that immunosuppressive therapy can modify the natural history of the hepatic disease due to the increase in viral replication and the potential hepatotoxicity of these drugs (4). The impact on patient survival in HBV-positive recipients does not become apparent until eight years after transplantation (5), when end-stage liver failure is responsible for death in 8 to 28% of these patients (1).

Parfrey and co-workers (6,7) showed that 82% of HBV-positive renal transplant patients followed up for 83 months presented

progressive hepatic disease and a worse outcome compared to patients maintained on dialysis. Based on these findings, these investigators do not advise renal transplantation. In addition, Rao et al. (8) do not recommend renal transplantation, particularly in cases with histological evidence of chronic liver disease.

In contrast, other studies have demonstrated that, despite persistent viral replication in HBV-positive renal transplant patients, no difference was observed in graft or patient survival in 10 years of follow-up (9,10). Thus, these groups advise renal transplantation for HBV carriers in the absence of cirrhosis or active chronic hepatitis.

Some antiviral therapies such as interferon-α used for the treatment of HBV infection have been shown to be effective in approximately 37% of cases (11). However, interferon-α presents immunomodulatory effects and thereby can induce rejection episodes in transplant patients (12). In addition, treatment with interferon-α has a high cost, limiting its use.

Recently, the action of lamivudine in blocking reverse transcriptase of HBV has been recognized (13). Lamivudine is a synthetic nucleoside analog with a potent action on HBV replication, thus decreasing the progression of liver disease (14). Unlike interferon-α, lamivudine does not present an immunomodulatory effect and therefore could be used in renal transplant patients with HBV infection. The aim of the present study was to evaluate the effectiveness and safety of lamivudine therapy in HBV-positive renal transplant patients.

Six HBV-positive renal transplant patients were included in the present open label, uncontrolled prospective study. Patients, 4 males and 2 females, were submitted to renal transplantation at the Nephrology Clinic of Hospital Beneficência Portuguesa, São Paulo, SP, Brazil. Four patients received kidneys from living related donors and two from cadaveric donors. The patients had a mean age of 37.6 ± 4.8 years and remained on dialysis treatment for 18.2 ± 4.9 months. The Hospital's Ethics and Research Committee approved the study and all patients gave informed consent to participate.

Patients were started on antiviral therapy 25.7 ± 17.5 months after renal transplantation, receiving 150 mg/day lamivudine (Epivir®, Glaxo Wellcome, Rio de Janeiro, RJ, Brazil) orally. The treatment was maintained daily and patients were reevaluated after 6, 12, 18, and 24 months of follow-up. Two patients had liver biopsies at the beginning of this study that showed active chronic hepatitis.

The triple-drug regimen for renal transplantation consisted of cyclosporine neoral, azathioprine and prednisone. In two patients azathioprine was later replaced by mycophenolate mofetil.

The laboratory monitoring included HBV DNA levels, serologic viral markers (HB surface antigen and HB envelope antigen, HBsAg, HBeAg, respectively), liver enzymes (alanine aminotransferase, ALT; gamma-glutamyl transferase, γ-GT), serum creatinine, and blood cyclosporine levels (reference values: ALT <40 U/l; γ-GT <80 U/l; creatinine <1.2 mg/dl). HBV viral load was quantified by the Branched DNA Amplification method, as used by the Corning-Nichols Institute (San Francisco, CA, USA). With this method, 56 x 10^6 Eq/ml is equal to 200 picograms of HBV DNA/ml. Values lower than 0.7 ± 10^6 Eq/ml are undetectable. The HBV serologic markers were analyzed by enzyme-linked immunoassays for HBsAg and HBeAg. The hepatic enzymes ALT and γ-GT were measured by quantitative determination of their catalytic activity in plasma. Serum creatinine concentration was determined by the Jaffe method. Blood cyclosporine levels were determined by polarized fluorescence immunoassay.

The serum biochemical and serologic markers in the course of treatment with lamivudine are summarized in Table 1. The

change of HBV DNA was most prominent. In all cases, lamivudine therapy was associated with a rapid decrease in HBV DNA. Before lamivudine therapy, the quantitative test for HBV DNA showed levels of 12,270 ± 4,946 x 10^6 Eq/ml (ranging from 126 to 30,000 x 10^6 Eq/ml). After three months of therapy, HBV DNA decreased significantly to a mean level of 1.6 ± 0.5 x 10^6 Eq/ml (P = 0.004), being undetectable in four cases. By the 12th month the level became undetectable in all patients. By the 24th month of treatment with lamivudine one patient presented renewed HBV replication with HBV DNA at 526 x 10^6 Eq/ml, suggesting development of resistance to lamivudine (Table 2). By the 24th month of follow-up the mean HBV DNA was 88.25 ± 87.55 x 10^6 Eq/ml (Table 2). Resistance to lamivudine was also detected in another patient (2,353 x 10^6 Eq/ml) after 30 months of follow-up (data not shown).

HBsAg persisted in all patients despite lamivudine treatment. Seroconversion (positive to negative HBeAg) and development of antibody to HBeAg were achieved by only one patient after three months of treatment.

The baseline serum ALT was elevated (mean: 64 ± 24 U/l) before treatment with lamivudine. After three months of treatment mean serum ALT was 54 ± 23 U/l, remaining around this level throughout follow-up. The γ-GT levels were roughly the same throughout the study.

None of the patients showed elevation of serum creatinine during lamivudine therapy. Renal graft function remained stable during follow-up, without episodes of acute rejection. Furthermore, blood cyclosporine levels revealed no significant change. Lamivudine

Table 1. Laboratory evaluation of renal transplant patients with hepatite B virus (HBV) infection treated with lamivudine.

	T_0	T_3	T_6	T_{12}	T_{24}
HBV DNA (x 10^6 Eq/ml)	12,270 ± 4,946	1.6 ± 0.5*	1.1 ± 0.3*	0.7 ± 0*	88.3 ± 88
HBsAg+ (No. of patients)	6	6	6	6	6
HBeAg+ (No. of patients)	6	5	5	5	5
ALT (U/l)	64 ± 24	54 ± 23	43 ± 8	45 ± 14	40 ± 11
γ-GT (U/l)	53 ± 20	49 ± 20	35 ± 7	52 ± 22	54 ± 26
Creatinine (mg/dl)	1.2 ± 0.1	1.1 ± 0.1	1.0 ± 0.1	1.1 ± 0.1	1.2 ± 0.1
Cyclosporine (ng/ml)	190 ± 22	207 ± 16	199 ± 20	194 ± 28	185 ± 27

Data are reported as means ± SEM. T_0: before treatment; T_3: third month; T_6: sixth month; T_{12}: 12th month; T_{24}: 24th month. HBsAg and HBeAg: HB surface antigen and HB envelope antigen, respectively; ALT: alanine aminotransferase; γ-GT: gamma-glutamyl transferase.
*P = 0.0021 *vs* T_0 (ANOVA, Tukey post test).

Table 2. Hepatitis B virus (HBV) DNA in HBV-infected renal transplant patients treated with lamivudine.

	T_0	T_3	T_6	T_{12}	T_{24}
Patient 1	4,269 x 10^6	3.4 x 10^6	0.7 x 10^6	0.7 ± 0	0.7 x 10^6
Patient 2	4,800 x 10^6	0.7 x 10^6	0.7 x 10^6	0.7 ± 0	0.7 x 10^6
Patient 3	10,000 x 10^6	0.7 x 10^6	0.7 x 10^6	0.7 ± 0	0.7 x 10^6
Patient 4	30,000 x 10^6	0.7 x 10^6	0.7 x 10^6	0.7 ± 0	0.7 x 10^6
Patient 5	126 x 10^6	2.7 x 10^6	1.1 ± 0.4	0.7 ± 0	526 x 10^6
Patient 6	24,400 x 10^6	1.1 x 10^6	1.1 ± 0.4	0.7 ± 0	0.7 x 10^6

T_0: before treatment; T_3: third month; T_6: sixth month; T_{12}: 12th month; T_{24}: 24th month.

was well tolerated by all patients with no significant adverse reactions.

The aim of treating a chronic HBV carrier with lamivudine is to suppress the viral replication before liver disease progresses and develops into cirrhosis. In the present study, lamivudine was effective in suppressing the replication of HBV in most of the transplant patients during the study period. After three months of treatment, HBV DNA decreased significantly and became undetectable in four patients. By the 12th month, all patients showed undetectable levels of HBV DNA. Similar results were also observed by Rostaing et al. (15), who revealed undetectable levels of HBV DNA after one month of treatment, in a study of six renal transplant patients receiving lamivudine. In another study, Jung et al. (16) treated six renal transplant patients who were HBV carriers. They were negative for HBV six weeks after the beginning of therapy with lamivudine, with the virus remaining undetectable during 7 to 14 months of follow-up.

In the present open trial, negative conversion of HBsAg was not observed in any patient, despite the reduction of HBV DNA. The persistence of HBsAg after treatment with lamivudine was also observed in other studies (15,16). This apparently paradoxical finding could be explained by taking into account the complex mechanism of HBV replication (17). Newly formed HBV can be produced after encapsulation of full length viral RNA, which is converted to DNA by the action of virion-associated reverse transcriptase. Alternatively, through the action of the virus-encoded reverse transcription, viral replication can be completed in hepatocytes, and viral DNA can become permanently established in the host cell. The latter is the main target of lamivudine. In fact, *in situ* hybridization studies have shown that the amount of HBV in hepatocytes remained the same after treatment with lamivudine (18). In the present study, HBeAg remained positive in five patients, indicating the presence of persistent viral replication.

The levels of ALT and γ-GT remained stable during the antiviral treatment, suggesting lack of hepatic reactivation. These results are consistent with data reported by Rostaing et al. (15) and Jung et al. (16).

Unlike interferon-α, lamivudine does not have an immunomodulatory effect. In our study, lamivudine treatment was not associated with rejection episodes. In addition, it did not affect the metabolism of cyclosporine, as evidenced by the lack of change in blood cyclosporine levels.

The appropriate dosage and duration of lamivudine treatment needs to be further evaluated in studies with larger groups of patients. Furthermore, longer therapeutic regimens can also be associated with emergence of resistant mutations (13).

Resistance to lamivudine was observed in two patients at 18 and 30 months of follow-up. Genotypic analysis of virus isolated from patients who developed HBV replication during lamivudine treatment suggested that two main mutations can confer resistance. One mutation results in a methionine to valine or isoleucine substitution in the YMDD motif of the catalytic domain of HBV polymerase. Another mutation results in a leucine to methionine substitution at position 528 upstream of the YMDD locus (19). The development of resistance should be suspected in patients who have breakthrough viral replication indicated by reappearance of serum HBV DNA after its initial disappearance. Beneficial effects of continued lamivudine treatment may still exist in patients with resistant mutants related to impaired replication ability of the mutants. This may account for low serum HBV DNA levels in patients with resistant mutants, and the rapid outgrowth of wild-type virus upon withdrawal of lamivudine. Another strategy for treatment of lamivudine-resistant HBV may be a combination with other drugs, such as adefovir (20).

Lamivudine was safe and effective in

blocking HBV replication for the 24 months of the study in most of the patients, without apparent interference with renal allograft function. However, combination of lamivudine with other antiviral agents should be also investigated to minimize the development of drug resistance.

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