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BRIEF REPORT

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Ribavirin therapy for hepatitis C infection following liver transplantation

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Abstract Hepatitis C infection following orthotopic liver transplantation may lead to progressive chronic graft dysfunction. In this study, seven liver transplant recipients with chronic allograft dysfunction due to hepatitis C infection (one acquired and six recurrent infections) were treated with oral ribavirin for 6 months. Symptoms of lethargy, nausea and anorexia improved in all patients within 2 weeks of starting the drug, with a fall in serum AST of at least 40 % by this time. Ribavirin-induced haemolysis was clinically significant in three patients, necessitating a reduction in the daily dose of ribavirin from 1.2 g to 0.2 g. Comparison of the pre- and post-treatment biopsy specimens in the four patients who tolerated the full dose of ribavirin and who had normal AST levels at the end of 6 months of treatment showed significant histological improvement with reduction in either lobular or periportal inflammation in all of the patients and a reduction in periportal fibrosis in one patient. HCV RNA remained detectable in serum in all of the patients at the end of the study.

Key words Hepatitis C, liver transplantation, ribavirin · Liver transplantation, hepatitis C, ribavirin · Ribavirin, liver transplantation, hepatitis C

Introduction

There is currently considerable interest in the management of hepatitis C (HCV) infection in the graft following liver transplantation. Nearly all patients transplanted for end-stage chronic HCV-related disease will have HCV RNA detectable in both serum and the liver post-transplant [3, 15], and a small number of patients may acquire HCV infection at the time of transplant, either from blood transfusion or from the donor liver [21]. Although the natural history of HCV infection of the liver graft tends to be mild [12], some patients can develop serious graft dysfunction. α Interferon is the most effective treatment for HCV in nontransplanted patients but does not appear to be very effective in allograft recipients. In a recent series only 9 % of patients responded with a fall in transaminases and none showed histological improvement [20]. α Interferon may also possibly precipitate rejection due to its immunostimulatory properties. The nucleoside analogue, ribavirin, has recently been reported to reduce transaminase levels and to have some effect in inhibiting HCV replication in nontransplanted hepatitis C-infected patients [2, 8, 13]. The pilot study reported here was designed to investigate the efficacy of short-course treatment with ribavirin in the treatment of HCV infection following liver transplantation.

Patients and methods

Liver transplant recipients with graft dysfunction of at least 6 months duration (AST at least twice the normal upper limit and histological evidence of chronic hepatitis) with evidence of active HCV infection were considered for this study, provided they had not received any other antiviral treatment in the preceding 6 months. Pregnant women or those at risk of pregnancy were ex-

cluded. Histological criteria were the presence of intralobular, piecemeal, or bridging necrosis, in association with a mononuclear portal or lobular infiltrate without vascular or bile duct change of cellular or ductopenic rejection. The degree of accompanying fibrosis or fatty change was also recorded. Active HCV infection was defined by the detection of HCV RNA in the serum using the reverse transcriptase polymerase chain reaction (RT-PCR) [4] with nested primers from the 5' noncoding region of the HCV genome [5]. In addition, serum was tested for anti-HCV before and after transplant using a second generation ELISA method (UBI, New York). CMV infection was excluded in all patients by a negative CMV antigen test in serum, negative white cell culture for CMV, unhelpful serology (negative CMV IgM or lack of four fold increase in IgG titre) and a nondiagnostic biopsy (including negative culture for CMV and negative immunostaining with monoclonal antibody). No patient had serological evidence of active HBV, HAV, HSV or EBV infection.

Oral ribavirin was administered in two divided doses – 1 g/day for adults less than 70 kg in weight and 1.2 g/day for patients heavier than this – for an initial period of 6 months. Any patient with a clinical or histological response was continued on ribavirin thereafter. Follow-up visits for symptom analysis and blood tests were performed at 1, 2, 4, 12, 24 and 52 weeks. If significant anaemia was detected (Hb < 100 g/l) together with evidence of haemolysis (reticulocytosis and reduced haptoglobins), the dose of ribavirin was reduced to 200 mg/day until haemoglobin had increased to greater than 100 g/l. Liver biopsy was repeated after 6 months of treatment. Histological assessment of the paired biopsy specimens was performed by an experienced liver histopathologist (BP) who was blinded as to the timing of the specimens. Each was scored according to the degree of portal, lobular and periportal inflammation [16], the presence and severity of fibrosis and the presence of lymphoid aggregates, bile duct damage and fatty infiltration.

Approval for this study was obtained from the King's Healthcare Research Ethics Committee in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. All patients gave informed consent prior to inclusion in this study.

Results

Between February and October 1992, out of a total of 316 recipients currently being followed at the Liver Transplant Clinic at Kings College Hospital, only 7 patients with chronic hepatitis fulfilled the criteria for the study. The three men and four women had a mean age of 52 years and were between 8 and 33 months after transplant (median 10 months). Six patients had been transplanted for known HCV-positive chronic liver disease and all six remained seropositive for anti-HCV antibody (UBI) post-transplant. The remaining patient (case 7), who was transplanted for primary biliary cirrhosis, was anti-HCV antibody-negative prior to transplantation and HCV RNA was not detectable by PCR in the explanted liver. She developed acute graft dysfunction (AST 260 U/l) 7 months after transplantation. Liver biopsy showed acute lobular hepatitis and HCV RNA was detected by RT-PCR in both serum and the liver, confirming the diagnosis of acquired HCV infection of the graft.

Primary immunosuppressive therapy after transplantation consisted of triple therapy with cyclosporin, azathioprine and prednisolone in five patients, one of whom changed to FK 506 and prednisolone as rescue therapy for intractable rejection 3 months after transplant (1 year prior to enrollment in this study). The other two patients received FK 506 and prednisolone as primary immunosuppression. All patients were taking a low maintenance dose of prednisolone (2.5–7.5 mg/day), but this drug was withdrawn following the diagnosis of HCV disease in the graft and prior to entry into this study. No patient subsequently developed acute or chronic rejection and no further changes in the immunosuppressive regimen were made during the study other than adjustment of cyclosporin and FK 506 doses to keep drug levels within the therapeutic range (cyclosporin whole blood level of 120–200 μg/l; FK 506 whole blood level of 10–20 μg/l).

Symptoms of anorexia, nausea and lethargy, present in all patients since the onset of graft dysfunction, were significantly improved within 2 weeks of starting ribavirin. The initial symptoms recurred rapidly in three patients following withdrawal of the drug. Pretreatment serum AST levels ranged between 94 and 240 U/l (median 130 U/l); levels began to fall soon after starting ribavirin therapy and had decreased to 50 % of the pretreatment value in all seven patients after only 2 weeks. By the end of 3 months, the AST level was normal in the four patients who tolerated the initial dose of ribavirin and it remained normal thereafter. In the remaining three patients, the dose of ribavirin was reduced after 1 month of treatment on account of haemolysis. In two of these patients (cases 4 and 6), the AST levels rapidly climbed again to pretreatment levels, whilst in the third patient (case 7) the AST increased slowly. Serum AST remained elevated in these three patients throughout the study (Fig. 1).

Comparison of the pre- and post-treatment liver biopsy specimens for each patient showed significant resolution in periportal and lobular inflammation in the four patients who had remained on full-dose ribavirin for the full period of treatment, with a return to normal in two patients (cases 1 and 2). In one patient (case 5), the degree of periportal fibrosis appeared less in the post-treatment biopsy. In contrast, none of the post-treatment biopsies of the three patients who were maintained on reduced doses of ribavirin demonstrated any histological improvement.

The four patients who had evidence of histological improvement after 6 months continued taking ribavirin. After 1 year of ribavirin treatment, serum AST remained normal in all four of these "responders" (Fig. 1). The three patients without histological improvement stopped ribavirin therapy after 6 months and AST has remained abnormal in all three since (Fig. 1). One of these "nonresponders" had a further liv-

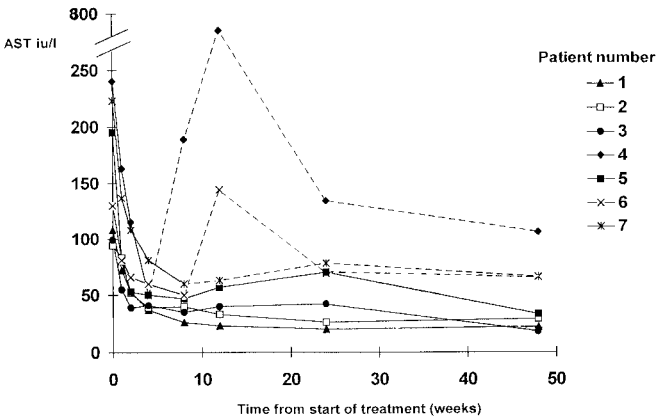

Fig. 1 Serial AST levels of seven liver transplant recipients with chronic HCV infection during ribavirin therapy. *Solid lines* demonstrate the effect of full-dose treatment. In comparison, *broken lines* show the effect of dose reduction in three patients who developed severe haemolysis. Normal AST < 50 IU/l.

er biopsy 1 year later that demonstrated progression to cirrhosis.

RT-PCR for HCV RNA in serum was repeated for all patients after 6 months of treatment and remained positive in all seven.

Some evidence of haemolysis was found in all patients with a fall in haemoglobin of between 12 % and 24 % (median 20 %) from the pretreatment level after 1 month of ribavirin treatment. Absolute reticulocyte count increased from normal (< 150×10^9/l) to between 164 and 356×10^9/l (median 279) and serum haptoglobins were reduced (< 0.5 g/l) in all patients. The haemolysis was clinically significant in three patients (cases 4, 6 and 7), with haemoglobin levels of 90, 70 and 82 g/l, respectively, after 1 month of treatment. Following the reduction in the dose of ribavirin from 1 or 1.2 g/day to 200 mg/day, haemoglobin levels rose to over 100 g/l in all three patients. Attempts to increase the dose further failed because of the recurrence of severe haemolysis. The other four patients (cases 1, 2, 3 and 5) tolerated the full dose of ribavirin for the 12-month treatment period without developing symptomatic haemolytic anaemia.

The serum bilirubin level was normal (< 25 μmol/l) in all patients prior to treatment and remained normal in four patients throughout the study. It became elevated in the three patients with significant haemolysis after 2–4 weeks of therapy (to 28, 36 and 43 μmol/l) due predominantly to an increase in unconjugated bilirubin, together with other evidence of haemolysis. The bilirubin level fell to normal values in all three patients following a reduction in the dose of ribavirin. The median serum alkaline phosphatase level prior to treatment was 210 U/l, ranging between 86 and 320 U/l (normal < 120 U/l). At the end of 6 months, this had fallen to 110 U/l, ranging between 60 and 190 U/l.

Discussion

Ribavirin (1-β-D-ribofuranosyl-1,2,4-triazole-3-carboxamide) is a noninterferon-producing nucleoside analogue that inhibits a wide range of RNA and DNA viruses both in vitro and in vivo [9–11, 14, 17–19]. Ribavirin produces its antiviral effects through three separate mechanisms: by depletion of the intracellular pool of GTP (cofactor in several essential viral enzyme pathways) through the direct inhibition of inosinate dehydrogenase; by the interruption of viral messenger RNA synthesis by incorporation of ribavirin metabolites into viral (but not host) mRNA; and, finally, by direct inhibition of the virus-coded RNA polymerases used to prime and elongate viral mRNA.

The most frequently reported side effect of oral ribavirin therapy has been a dose-related haemolytic anaemia [2, 8, 13]. In this present study, although the haemolysis was detectable in all of the patients, four of the seven tolerated the full dose of the drug, and in the remaining patients haemolysis was rapidly reversible following a reduction in the dose. The mechanism of the haemolysis is unknown but is probably related to the rapid accumulation of the drug inside red cells (concentrations of 50–100 times plasma concentrations). Unlike α-interferon, ribavirin does not enhance the immune response. In fact, there is evidence that ribavirin may improve graft (skin and heart) survival in combination with the standard immunosuppressive drugs [7, 11, 17].

Although previous studies in nontransplanted patients with hepatitis C infection have reported a significant reduction in transaminases during ribavirin therapy, repeat biopsies after 6 months failed to show histological improvement in any patient [2]. Our study suggests that this drug may be more effective following liver transplantation: seven patients with both clinical and histological evidence of chronic HCV liver disease had rapid improvement in both symptoms and transaminases following the administration of ribavirin therapy and, more importantly, histological improvement was demonstrated in all four cases who were maintained on the full dosage.

In nontransplanted patients, there is evidence that the titre of HCV RNA in serum may parallel the severity of liver disease [6], which would suggest that HCV-induced liver injury is produced by the direct cytocidal effects of the virus rather than by immunological mechanisms. Immunosuppression enhances HCV replication, and very high serum levels of HCV RNA are found in liver transplant recipients with chronic HCV infection [1]. This increased viral load may be one of the factors responsible for the rapidly progressive liver disease observed in some patients following liver transplantation.

In nontransplanted patients with HCV infection, although serial semiquantitative measurement of serum

HCV RNA levels demonstrated a reduction in viral load in many patients taking ribavirin [2], serum HCV RNA remained detectable in all patients. Serum HCV RNA levels, like serum transaminases, rapidly returned to pretreatment levels after treatment was stopped [2, 8]. In our study, all patients remained viraemic despite both biochemical and histological responses, and a rapid relapse in symptoms and elevation of AST occurred in all three patients in whom ribavirin was temporarily withdrawn because of haemolysis.

Although single drug therapy with ribavirin may suppress HCV replication, it does not eradicate the viraemia. Prolonged treatment courses (probably lifelong) with ribavirin alone, or perhaps combination therapy with other antiviral agents, may be necessary to prevent progression of HCV-related graft disease after liver transplantation.

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