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Early use of tacrolimus as rescue therapy for refractory liver allograft rejection

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Abstract The aim of this study was to compare two different periods of tacrolimus rescue therapy for intractable rejection. From January 1992 to May 1996, 140 liver transplants (LTx) were performed in our hospital under cyclosporine A-based immunosuppression. Twenty-four (17.1%) patients were switched to tacrolimus because of chronic rejection, steroid-resistant rejection or cholestasic hepatitis C recurrence. Mean follow-up was 21 months (range 12–56 months). In the first period (January 1992–March 1994), conversion to tacrolimus was indicated later, after unsuccessful repeated rejection therapy. In the second period (April 1994–May 1996), conversion to tacrolimus was indicated early, immediately after unseccessful rejection therapy or directly at the moment of diagnosis with no further treatment. Eleven of 54 LTx were treated with tacrolimus in period 1 (20.3%), and 13 of 86 LTx in period 2 (15.1%). Only 4 of 11 (36.6%) grafts converted were rescued during the first period, while 11 of 13 (84.6%) were rescued in the second ($P < 0.03$). Patients in the first period received more courses of steroids than those of the second (1.7 ± 0.7 vs 0.9 ± 0.7, $P < 0.02$). Furthermore, six patients received one or two courses of OKT3 in period 1 while only one received one course in period 2 ($P < 0.03$). Preconversion mean bilirubin levels of patients in the first period were higher than those in the second (15.9 ± 7.3 mg/dl vs 9.7 ± 5.8 mg/dl, $P < 0.05$). Preconversion mean bilirubin levels of 6.8 ± 5.4 mg/dl and 21.8 ± 18.5 mg/dl were observed in patients with successful and unsuccessful tacrolimus rescue therapy, respectively, independent of the treatment period ($P < 0.05$). Mortality rates were higher in the first period than in the second (82% versus 23%; $P < 0.02$). In conclusion, conversion to tacrolimus as rescue therapy for intractable rejection or cholestasic hepatitis C recurrence is an efficacious alternative, particularly when tacrolimus is initiated early.

Key words Tacrolimus · Refractory rejection · Hepatitis C virus · Liver transplantation

Introduction

Uncontrolled rejection or certain allograft dysfunctions under cyclosporine A (CyA)-based immunosuppression continue to present considerable problems despite novel strategies designed to optimize the use of CyA [1]. Current modalities available for treating steroid-resistant rejection episodes or chronic rejection under CyA-based immunosuppression are limited and require retreatment with anti-lymphocyte regimens, which increase the risk of infection, particularly by cytomegalovirus [2]. Furthermore, patients with recurrence of

hepatitis C virus (HCV) and duct or ductular damage associated or not with a certain degree of ductopenia may show difficulties in immunosuppressive CyA management [3]. Tacrolimus is a new immunosuppressor agent with mechanisms of action similar to those of CyA, but is significantly more potent [2, 4]. Different studies in the United States [5–7] and Europe [8, 9] have proven the efficacy of tacrolimus for reversal of refractory acute cellular rejection and early chronic rejection, under optimal standard CyA and adjuvant immunosuppression. Frequently, this drug is started in advanced allograft dysfunctions, achieving partial or no response [5, 6]. Our first experience with tacrolimus as rescue therapy for patients with refractory liver graft rejection was not satisfactory and consequently the protocol of conversion was changed [10, 11].

The aim of this study was to compare two different periods of tacrolimus rescue therapy for intractable rejection.

Materials and methods

From January 1992 to May 1996, 167 liver transplant (LTx) were performed in our hospital. One-hundred and forty patients were maintained under CyA-based immunosuppression and 27 under tacrolimus as a main immunosuppressive drug, forming part of a multicenter trial. These patients were excluded from the study. Twenty-four (17.1%) patients were switched to tacrolimus after CyA-based immunosuppression failed, mainly because of uncontrolled rejection or duct damage in the context of cholestasic HCV recurrence.

Mean follow-up was 21 months (range 12–56 months).

Immunosuppression

The immunosuppression protocol was changed during the study period. The first 115 patients were maintained by CyA (Sandimmun) and prednisone (P). Induction therapy consisted of 1 g of methylprednisolone i.v. before revascularization, followed by 200 mg/day P decreasing to 20 mg/day over 6 days. CyA was begun in the postoperative period at 2 mg/kg per day by continuous intravenous infusion, with the dose increasing to a steady-state level of 300–350 ng/ml determined by enzyme immunoassay during the first week. The remaining patients were maintained by a new oral microemulsion formulation of CyA (Sandimmun Neoral) and prednisone. CyA Neoral was begun in the postoperative period at 10 mg/kg per day by nasogastric probe, with the dose increasing to achieve a trough level of 250–350 ng/ml determined by enzyme immunoassay during the first week.

Acute rejection episodes were treated in all patients with a 3-day course bolus of 1 g methylprednisolone. If rejection persisted, a 14-day course of 5 mg/day of OKT3 was given mainly in patients from the first period (January 1992–March 1994).

Steroid-resistant rejection

Steroid-resistant rejection was defined whenever one or more histopathological signs of acute rejection [12] persisted in allograft biopsy after one 3-day course of methylprednisolone [13].

Chronic rejection

Chronic rejection was defined by the absence of interlobular and septal bile ducts in 50% or more of portal tracts in liver biopsy. Obliterative vasculopathy involving large and medium-sized arteries was considered a non-obligatory but supportive feature [12].

Duct damage in cholestasic recurrence of HCV

Cholestasic hepatitis was defined by persistent increases in two or more of the following values: bilirubin, alkaline phosphatase, and gamma-glutamyl transpeptidase, for at least 3 weeks. Liver biopsy showed graft hepatitis related to HCV reinfection. Duct damage was defined by periductal infiltrate of lymphocytes, occasional mononuclear cells adjacent to basement membrane or within the duct epithelial wall, and possible slight foci of duct epithelial loss [3].

Protocol for conversion

Indications for conversion to tacrolimus were: steroid-resistant rejection, chronic rejection, duct damage in the context of cholestasic HCV recurrence, and repeated episodes of acute rejection in one patient with malabsorption of CyA (Sandimmun, classical formulation). Doppler ultrasound of the allograft was always performed to rule out biliary or vascular complications prior to tacrolimus conversion. In addition, biopsies of the allograft were performed in all cases to assess diagnosis as part of the evaluation for tacrolimus conversion.

The protocol of conversion from CyA to tacrolimus changed during the study.

First period

In the first period (January 1992–March 1994), all patients received Sandimmun, the classical formulation. Conversion to tacrolimus was indicated later, after unseccessful repeated rejection therapy (at least two 3-day courses of bolus steroids or one 14-day course of OKT3).

Second period

In the second period (April 1994–May 1996), 5 of 13 patients received Sandimmun Neoral. Conversion to tacrolimus was indicated early, directly or immediately after unsuccessful rejection therapy (only one 3-day course of bolus steroids). During this period, patients with suspected or confirmed chronic rejection, or patients with ductopenia and/or duct damage in the context of cholestasic HCV recurrence, were converted early to tacrolimus, with no further treatment.

Tacrolimus was always started orally after withdrawal of the final the dose of CyA. Initial dosage was calculated at 0.05 mg/kg twice a day. Dosage adjustments were based on monitoring through serum tacrolimus levels to achive a 12-h trough level of 5–20 ng/ml determined by a monoclonal antibody technique and also by adjustment according to clinical and biochemical parameters. In patients with severe graft dysfunction, principally those with cholestatic hepatitis, the initial dosage must be lower than the conventional dosage owing to the slow metabolism of the drug by the allograft.

Table 1 Cases of conversion to tacrolimus (*HCC* hepatocellular carcinoma, *HCV* hepatitis C virus, *SRR* steroid-resistant rejection, *CyA* cyclosporine A, *NS* not significant)

	Period 1 (January 1992–March 1994)	Period 2 (April 1994–May 1996)	*P*
Number of transplants	54	86	
Original disease			NS
Postnecrotic cirrhosis	22	37	
HCC/HCV (+)	10	16	
Cholestasic disease	3	4	
Alcoholic cirrhosis	10	12	
Retransplantation	5	6	
Other	4	4	
Conversion to tacrolimus	11 (20.3%)	13 (15.1%)	NS
Chronic rejection (concomitant HCV)	7 (4)	3 (1)	
SRR	3	6	
Cholestasic HCV	0	4	
Malabsorption	1	0	
Age (years, mean ± SD)	48 ± 11.1	52.2 ± 11.6	NS
Male/female	4/7	6/7	NS
CyA/CyA Neoral	11/0	8/5	0.02

Table 2 Preconversion immunosuppression and liver function (*Bb** bilirubin)

	Period 1 (January 1992–March 1994)	Period 2 (April 1994–May 1996)	*P*
Courses of steroids	1.7 ± 0.7	0.9 ± 0.7	< 0.02
Patients with OKT3	6 of 11 (54.5%)	1 of 13 (7.6%)	< 0.03
Preconversion Bb* (mg/dl)	15.9 ± 7.3	9.7 ± 5.8	< 0.05

Evaluation of outcome

Outcome was evaluated in terms of response or non-response. Response was assessed as positive if cellular rejection was reversed or if progression of bile duct loss in chronic rejection was at least interrupted, thereby improving liver function, at least after 3 weeks follow-up of conversion. Non-response was assessed if allograft dysfunction (defined as more than 50% over normal values of liver function tests) persisted after 3 weeks of tacrolimus therapy. Repeated biopsies were performed to assess unresponsiveness when the clinical course showed no improvement.

Statistical analysis

Quantitative data were analyzed using Student's *t*-test or the Mann-Whitney U test and categoricals by Pearson chi-squared or Fisher's exact test. The difference was considered statistically significant when $P < 0.05$. Data are shown as mean values ± SD.

Results

Table 1 shows the circumstances of patients in both periods. Eleven of 54 LTx were treated with tacrolimus in period 1 (20.3%) and 13 of 86 LTx in period 2 (15.1%). No statistical significance was found in either group regarding age, diagnosis, sex, and indication for LTx, with both groups being comparable, except in immunosuppression.

Preconversion immunosuppression and liver function

Prior to entry into the study, patients in the first period received more courses of steroids than those in the second (1.7 ± 0.7 versus 0.9 ± 0.7, $P < 0.02$). Furthermore, five and one patients received one and two courses of OKT3, respectively, in period 1, while only one received one course in period 2 ($P < 0.03$). Although the differences were not statistically significant, the median interval between diagnosis of liver dysfunction and initiation of tacrolimus was higher in the first period than in the second (36.09 ± 27.1 days versus 20 ± 62 days, $P < 0.8$; Table 2).

Preconversion mean bilirubin levels of patients in the first period were significantly higher than those in the second (15.9 ± 7.3 mg/dl versus 9.7 ± 5.8 mg/dl, $P < 0.05$; Table 2). Preconversion mean bilirubin levels of 6.8 ± 5.4 mg/dl and 21.8 ± 18.5 mg/dl were observed in patients with successful and unsuccessful tacrolimus rescue therapy, respectively, independent of the treatment period ($P < 0.05$).

Effect of conversion to tacrolimus

Only 4 of 11 (36.6%) grafts converted to tacrolimus were rescued during the first period, while 11 of 13

Table 3 Mortality and causes of graft failure (*CMV* cytomegalovirus)

	Period 1 (January 1992–March 1994)	Period 2 (April 1994–May 1996)	*P*
Mortality	81 %	23 %	< 0.02
Causes of death			
Sepsis	2	1	
Multiorgan failure	4		
CMV disseminated	1		
Tumor recurrence	1		
Pulmonary embolism	1		
Aspergillosis		1	
HCV recurrence		1	

(84.6 %) converted grafts were rescued in the second ($P < 0.03$).

Mortality and causes of graft failure

Mortality rates were higher in the first period than in the second (82 % vs 23 %, $P < 0.02$). Twelve patients remain alive, with a mean survival time of 25 ± 16 months.

Period 1

Six patients with chronic rejection died during follow-up. Two progressed to graft failure despite tacrolimus treatment and required retransplantation. One died immediately after surgery from unrelated causes and the other had a successful retransplant although he died some weeks after retransplant due to recurrence of hepatitis B and chronic rejection. The remaining four patients died from rejection-related causes, two of them while on the waiting list for retransplant. Two patients with steroid-resistant rejection died from rejection-related causes and the patient with malabsorption of CyA died from tumor recurrence (Table 3).

Period 2

One patient with chronic rejection died during follow-up. Another patient progressed to graft failure despite tacrolimus treatment and had a successful retransplant under tacrolimus therapy. One patient with steroid-resistant rejection died from rejection-related causes during follow-up, another with severe HCV recurrence died despite tacrolimus therapy (Table 3).

Discussion

Since its introduction in the 1980s, CyA has become the mainstay of most immunosuppressive regimens in liver transplantation. Despite the development of novel treatment strategies involving CyA, certain disadvantages associated with its use have persisted. The incidence of rejection episodes, even using multiple drug regimens based on CyA, remains high (30–50 %) [14]. Although the incidence of chronic allograft rejection in liver transplantation is low compared with that of kidney or heart allografts, retransplantation remains the main treatment [15].

We started using tacrolimus as a rescue therapy in patients with chronic rejection as an alternative therapy to retransplantation [11]. HCV reinfection is common in patients transplanted for HCV cirrhosis, and causes chronic graft hepatitis in many cases. A major diagnostic and management problem arises when chronic rejection appears in patients with chronic hepatitis and it is often difficult to assess whether chronic rejection or HCV hepatitis is the prevailing problem. In the first period, poor results were probably achieved because tacrolimus was started in end-stage chronic rejection and four of these patients had severe HCV recurrence [11]. Furthermore, in the first period, patients with steroid-resistant rejection were converted to tacrolimus because rejection was refractory to high doses of steroids and OKT3.

Severe HCV recurrence has been a further cause of conversion. It is difficult to make a correct diagnosis in patients with hepatitis C, particularly when liver dysfunction occurs early after transplant [3], since recurrence of HVC may be confused with rejection. Furthermore, these patients may present cholestasic hepatitis [16] with duct or ductular damage detected in liver allograft biopsy, mimicking the vanishing bile duct syndrome [3, 17, 18]. In the first period, these patients were aggressively treated with high doses of steroids because rejection appeared to play a role in liver dysfunction. Patients lost the graft owing to adverse events secondary to overimmunosuppression or recurrence of viral disease and none could be converted to tacrolimus. In the second period, these patients were rapidly converted to tacrolimus because certain immunological mechanisms may play a role in cholestasis pathogenesis. In fact, this form of hepatitis C is unusual in the native liver. An initial good response was obtained as far as im-

provement of cholestasis was concerned, although chronic, active allograft hepatitis persisted. One of the patients died from HCV recurrence after 1 year of the new treatment.

One patient suffered repeated acute rejection episodes with low CyA levels due to malabsorption, despite a high CyA dosage. The problem was completely resolved with tacrolimus and is currently rare with the use of Neoral CyA [19].

It can be observed that patients converted in period 1 show clinico-biological data which suggest advanced allograft dysfunction compared with those converted in the second period. These findings could explain the low rate of responsive patients observed with the use of the first protocol compared with the second. In fact, all patients rescued with tacrolimus presented significantly lower bilirubin levels than those in whom non-responsiveness was shown, regardless of the conversion protocol used. Although differences were not significant, the interval from diagnosis to tacrolimus conversion was shorter in the second period than in the first.

Mortality in our patients was imputable mainly to effects secondary to overimmunosuppression. These findings were particularly significant in the first period.

Our results concur with those of reports where the majority of patients responsive to tacrolimus were those in whom dysfunction was ascribable to steroid-resistant rejection and a bilirubin level less than 10 mg/dl [5, 7]. Effectiveness of conversion to tacrolimus in chronic rejection seems to be poor compared with steroid-resistant rejection, particularly in patients with concomitant hepatitis C reinfection [5]. According to our results, we believe that if conversion is performed early in patients with moderate liver dysfunction, ductopenia or histopathological signs of acute rejection may be reversed. Patients with hepatitis alone fared poorly [5].

In conclusion, conversion to tacrolimus as rescue therapy for steroid-resistant acute allograft rejection, chronic rejection or for patients with HCV recurrence and duct damage present in biopsy is an efficacious alternative, particularly when tacrolimus is initiated early. If patients show end-stage chronic rejection, early retransplantation must be indicated.

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