Transpl Int (1996) 9: 23–31

ORIGINAL ARTICLE

Sven Jonas
Wolf Otto Bechstein
Hans-Peter Lemmens
Norbert Kling
Onnen Grauhan
Hartmut Lobeck
Peter Neuhaus

Conversion to tacrolimus after liver transplantation

Received: 3 March 1995
Received after revision: 8 June 1995
Accepted: 13 June 1995

S. Jonas (✉) · W. O. Bechstein · H.-P. Lemmens
N. Kling · O. Grauhan · P. Neuhaus
Chirurgische Klinik,
Virchow Klinikum,
Humboldt Universität,
Augustenburger Platz 1,
D-13353 Berlin, Germany
Fax: + 49 30 4505 2900

H. Lobeck
Institut für Pathologie,
Virchow Klinikum,
Humboldt Universität,
Augustenburger Platz 1,
D-13353 Berlin, Germany

Abstract We have reviewed our experience with conversion to tacrolimus after 435 liver transplantations. Tacrolimus was administered as a rescue agent in 33 patients until October 1993. Indications for rescue therapy were: cholestatic forms of severe, steroid-resistant cellular rejection ($n = 8$), OKT3-resistant cellular rejections ($n = 6$), cellular rejections in patients suffering from cyclosporin malabsorption ($n = 4$), late onset cellular rejections ($n = 4$), early chronic rejections ($n = 3$), and chronic vascular or ductopenic rejections ($n = 8$). Response was evident in 29 of the 33 patients (88 %), whereas 4 patients (12 %) were nonresponsive. Patient and graft survival were 76 % and 70 %, respectively. Graft loss with or without patient death occurred in three of eight patients suffering from severe, steroid-resistant cellular rejection, in two of six patients with OKT3-resistant cellular rejections, and in five of eight patients undergoing chronic rejection. In severe steroid-resistant cellular rejection, successful tacrolimus rescue therapy corresponded to a significantly lower total serum bilirubin than unsuccessful therapy (12.0 ± 5.6 mg% vs 29.7 ± 5.9 mg%, $P < 0.05$). We conclude that tacrolimus rescue therapy is a safe and efficient alternative for high-risk cases that do not respond to conservative treatment. In severe, steroid-resistant cellular rejection and in chronic ductopenic rejection, conversion to tacrolimus is beneficial only in a limited number of cases. A predictive parameter, which total serum bilirubin may prove to be in severe, steroid-resistant cellular rejection, is needed to select those cases that might benefit more from retransplantation than from conversion to tacrolimus.

Key words Liver transplantation, FK506 conversion · Liver transplantation, tacrolimus conversion · FK506, liver transplantation, conversion · Tacrolimus, liver transplantation, conversion · Conversion, liver transplantation, FK506

Introduction

In liver transplantation, rejection rates of 40 %, along with 1-year survival rates of 90 %, are approached by programs employing synergistic quadruple regimens in which triple protocols are supplemented by antithymocyte globulin (ATG) [1, 11] or monoclonal anti-interleukin 2 receptor antibodies (anti-IL2R) [20, 23]. However, rejection continues to be one of the most common causes of graft loss, and an increased susceptibility to infection remains a frequent sequela of antirejection therapy. The most widely used agent in steroid-resistant rejection thus far has been OKT3, a monoclonal antibody that blocks the cytotoxicity-mediating CD3 determinant of the T-cell receptor. Though effective at reversing even late onset steroid-resistant rejection episodes [3,

27], its application is limited by an occasionally dramatic first-dose reaction, as well as by an increased incidence of serious opportunistic infections and lymphoproliferative disorders [25, 32, 34].

The naturally occurring macrolide tacrolimus was introduced in clinical immunosuppressive trials after solid organ transplantation by the Pittsburgh group, which has since elucidated its role as a rescue agent in cases of otherwise intractable rejection [33]. Conversion to tacrolimus appeared all the more tempting as its inherent risks, particularly nephrotoxicity and neurotoxicity, were mostly reversible and could be significantly reduced by oral instead of intravenous administration [7, 31]. The marked ability of tacrolimus to reverse ongoing rejection, despite evidence of ductopenic changes, was most pronounced when patients were converted during an early stage [8]. In fact, response rates of 60 %–70 % have been reported, while other reviews seem to indicate that only a subgroup of patients with clinically manifest chronic ductopenic rejection permanently benefit from it [13, 15, 36]. These data are also reflected in pediatric liver transplantation [5, 24]. Parameters predicting outcome after a switch to tacrolimus are not available, although evidence is emerging that excessive serum bilirubin levels might be predictive of nonresponse [4, 35].

In this report, we describe the outcome of 33 liver transplant recipients with different indications who were switched from cyclosporin (CyA)-based immunosuppressive regimens to tacrolimus rescue therapy.

Materials and methods

Patient selection

From September 1988 to October 1993, 435 liver transplantations were performed in 401 patients at our institution. In 322 transplantations, immunosuppression consisted of CyA-based immunosuppressive regimens. As part of different trials, a total of 113 patients received tacrolimus in order to evaluate its properties as a primary immunosuppressive agent. Between May 1990, when it first became available to our center, and October 1993, tacrolimus was administered as a rescue agent to 33 patients. Conversion from CyA-based immunosuppressive regimens to tacrolimus was implemented after informed consent had been obtained. The course of the patients was followed in the clinic during the first 4 weeks post-transplantation, or on a routine, clinical and outpatient basis later on.

Liver transplantation

Grafts were preserved almost exclusively in Belzer's University of Wisconsin solution. In two cases each, Euro-Collins and Bretschneider's HTK solutions were used. The surgical procedure was performed using a standardized technique comprising a veno-venous bypass and completion of all four vascular anastomoses prior to reperfusion. In all but 38 cases, which required a biliodigestive anastomosis due to the underlying disease, the biliary reconstruction was performed as a side-to-side choledocho-choledochostomy [21].

Primary immunosuppression

Primary immunosuppressive protocols consisted either of conventional triple therapy, of our standard quadruple drug induction regimen entailing an antithymocyte globulin preparation (ATG; Fresenius, Bad Homburg, Germany) [28], or of another sequential quadruple drug protocol using a monoclonal anti-interleukin-2 receptor antibody (BT563; Biotest, Dreieich, Germany) [20].

Except for ATG or BT563 treatment, an almost identical immunosuppressive regimen was applied, irrespective of the primary protocol group. CyA was started after surgery in a parenteral dose of 1–2 mg/kg body weight (BW) twice a day. If the clinical course and protocol cholangiography on post-transplant day 5 allowed capping the T-tube drainage, CyA was switched to an oral intake of 5 mg/kg BW twice a day. Subsequent dosing was adjusted according to whole blood levels, aiming at between 600 and 900 ng/ml, as measured by a polyclonal FPIA (TDX assay, Abbott). Methylprednisolone was given prior to reperfusion and directly after transplantation in a dose of 500 mg i.v. each time. Prednisolone was begun in a single oral dose of 1 mg/kg BW, tapered to 20 mg/day for the 1st month. Azathioprine was started as parenteral administration of 25 mg/day until 1 week after transplantation; on post-transplant day 7 the dosage was increased to 1–2 mg/kg BW orally. Intake was reduced or interrupted according to peripheral white blood counts.

ATG was started intra- or postoperatively in a dose of 5 mg/kg BW per day and given for 7 days in a continuous infusion for 6 h. BT563 was administered i.v. for 12 days in a daily dose of 10 mg.

Rejection episodes

Rejection was suggested by a deterioration in liver function (changes in total bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (AP), or γ-glutamyl transferase (γ-GT) concentrations) or by the appearance of clinical signs (fever, jaundice, pruritus, ascites, or a scant production of light bile). Whenever rejection was suspected, a liver biopsy was obtained for confirmation of the diagnosis [18]. Doppler ultrasound was performed, if indicated, to rule out hepatic artery or portal vein thrombosis. If suspicion of a vascular complication prevailed, diagnosis had to be confirmed by angiography. Bile leakage or biliary obstruction was excluded by cholangiography. Screening for infectious disorders entailed the collection of routine specimens for culture and microscopy, quantification of fungal and viral titers in blood, direct immunofluorescence for *Legionella* in blood and urine, and the search for cytomegalovirus (CMV) antigen in blood by the polymerase chain reaction (PCR) [29].

Core liver biopsies were obtained on post-transplant-day 7 or when rejection was suspected. The histopathological grading of acute or cellular rejections was classified as listed below [14, 20]:

Mild (grade I): mild periportal mononuclear infiltrate with minimal endothelialitis and focal duct damage involving less than 50 % of the bile ducts

Moderate (grade II): moderate periportal mononuclear infiltrate extending beyond portal field confines, or focal duct damage involving more than 50 % of the bile ducts

Severe (grade III): the same alterations as described for grade II plus severe injuries (arteritis, central ischemic damage, confluent necroses, paucity of bile ducts).

A diagnosis of chronic or ductopenic rejection depended largely on evidence of cholestasis with interlobular and septal duct loss. Other histologic criteria were the absence of findings concordant with viral hepatitis, obliterative arteriolar lesions, and portal tract fibrosis with linkage between central veins and portal triads. Dis-

tinction between early chronic and chronic rejection was based on the extent of lymphocytic bile duct damage or loss. A bile duct loss limited to less than 25 % of the sample triads without cholestasis or lobular changes was categorized as an early chronic rejection. Findings indicative of a chronic rejection were a bile duct loss of 50 % or more, lymphocytic damage in the remaining ducts, and hepatocanalicular cholestasis. The term "vanishing bile duct syndrome" (VBDS) was used for a ductopenic rejection involving 75 %–100 % of interlobular bile ducts together with a severe cholestasis, frequently accompanied by a foam cell arteritis [22]. For confirmation of bile duct loss, study of 20 portal tracts was required.

Depending on whether onset was prior to or after post-transplant day 90, cellular rejection episodes were classified as early or late onset rejections, respectively.

Treatment of rejection

Initial therapy consisted of a 3-day course of high-dose steroids, i.e., 500 mg/day methylprednisolone i.v. Steroid-resistant episodes were treated for another 5–10 days with 5 mg/day of monoclonal OKT3 antibody. Conversion to tacrolimus was applied in OKT3 nonresponders, or as soon as a chronic ductopenic rejection was suspected, irrespective of prior OKT3 treatment. A direct switch to tacrolimus for steroid-resistant cellular rejection was considered in late onset episodes and in patients with persistent CyA malabsorption in spite of a capped T-tube drainage. After gaining more experience with tacrolimus, we were more apt to implement a direct conversion for steroid-resistant cellular rejection even if the criteria mentioned were absent. Rejection episodes in these patients displayed a predominantly cholestatic pattern or were histopathologically classified as moderate-to-severe or severe.

Rescue therapy was started with continuation of oral steroids and administration of oral tacrolimus. Initial dosing ranged from 0.07 to 0.1 mg/kg BW twice a day. Further adjustments were related to toxicity and response or graft function.

Evaluation of outcome

Outcome was evaluated in terms of response or nonresponse and success or failure. Response was assessed as positive if a cellular rejection was reversed or if progression of bile duct loss in chronic rejection was at least interrupted, thereby improving liver function. Repeat biopsies were performed if the clinical course did not demonstrate an improvement. Success or failure was determined with regard to both patient and graft survival.

Statistical evaluation

Data are expressed as mean ± standard error of the mean. Comparisons between groups were made using the Wilcoxon rank-sum test for continuous variables and the chi^2-test for categorical variables. Differences were considered statistically significant when P values were below 0.05.

Table 1 Demographic data and primary immunosuppression in the study population

Patient characteristics	$n = 33$
Female/male	15/18
Age (years)	48.4 ± 10.7
Primary immunosuppression	
Triple therapie	5/29 (17.2 %)
Quadruple therapy (ATG)	21/191 (11.0 %)
Quadruple therapy (BT563)	7/102 (6.8 %)

Table 2 Indications for conversion to tacrolimus and outcome in terms of response and survival

Indication	n	Response		Survival	
		yes	no	yes	no
Chronic ductopenic rejection	8	5	3 (2)[a]	5 (2)[a]	3
Early chronic rejection	3	3		3	
OKT3-resistant cellular rejection	6	6		4	2
Steroid-resistant cellular rejection	8	7	1	5	3
Late onset cellular rejection	4	4		4	
Cellular rejection and CyA malabsorption	4	4		4	
Total	33	29 (88 %)	4 (12 %)	25 (76 %)	8 (24 %)

[a] Number of retransplantations

Results

Patient characteristics

Of the 435 patients transplanted between September 1988 and October 1993, 33 underwent tacrolimus rescue therapy after the drug became available to our center in May 1990. In 322 transplantations, CyA-based regimens had been administered as primary immunosuppression (triple $n = 29$; ATG $n = 191$; BT563 $n = 102$). Indications for transplantation in the 33 patients converted to tacrolimus included hepatitis C virus (HCV) disease ($n = 7$), hepatitis B virus (HBV) disease ($n = 4$), primary sclerosing cholangitis ($n = 4$), fulminant liver failure ($n = 4$), alcoholic cirrhosis ($n = 3$), Klatskin tumors ($n = 3$), and various other indications ($n = 8$). Table 1 depicts patient gender and age as well as the primary immunosuppression.

Indications for conversion

The secondary diagnoses triggering rescue therapy are shown in Table 2. The most common indications were chronic ductopenic rejections ($n = 8$), OKT3-resistant

cellular rejections ($n = 6$), and predominantly cholestatic or severe forms of cellular, steroid-resistant rejection ($n = 8$).

In the group of poor CyA uptake ($n = 4$), three patients had undergone liver transplantation and Whipple's procedure for Klatskin tumors, while this surgical approach had been performed in a total of 7 patients out of 322 with CyA-based regimens as primary immunosuppression [19]. In one patient, choledochojejunostomy for primary sclerosing cholangitis had been performed.

In almost all cases, initial treatment of rejection had consisted of a 3-day course of high-dose steroids, except for four patients who were converted directly to tacrolimus, two each in the group of CyA malabsorption and late onset cellular rejection. Cycles of OKT3 had already been administered in five patients with chronic rejection and in one patient each in the late onset and early chronic rejection groups.

Outcome

After a median follow-up of 2 years (25 ± 11 months), 29 of 33 patients (88 %) were responsive and 4 patients (12 %) were nonresponsive. Patient survival could be observed in 25 patients (76 %) and graft survival in 23 patients (70 %).

Nonresponsiveness was evident in three of eight cases in the chronic rejection group and in one of eight cases in the group of severe, steroid-resistant cellular rejections (Table 2). All early chronic rejections, OKT3-resistant cellular rejections, late onset cellular rejections, and all cellular rejections in patients with poor CyA uptake were effectively treated by conversion to tacrolimus.

There were eight lethal cases: in three of eight patients suffering from chronic ductopenic rejection, in three of eight patients in the severe, steroid-resistant cellular rejection group, and in two of the six OKT3 nonresponders (Table 2). In these patients, the average time between transplantation and the onset of rescue therapy and between the switch to tacrolimus and patient death was approximately 4 months (116 ± 145 days; range 11–454 days) and 3 months (89 ± 147 days; range 7–526 days), respectively. Two patients died while rejection episodes – one chronic ductopenic and one severe, steroid-resistant cellular – persisted. In six patients, rejection had ceased clinically. In one of these patient, who had suffered from chronic ductopenic rejection, histopathological features of rejection were ameliorated on autopsy when compared to pre-switch findings, though they had not disappeared completely. The liver graft of another patient who was converted due to chronic ductopenic rejection and who died of CMV pneumonia did not display rejection-like features but rather viral hepatitis without serological evidence of HBV or HCV infection. Autopsy was denied for one patient converted due to an OKT3-resistant cellular rejection. In the remaining four patients who had suffered from refractory cellular rejections, there was no longer evidence of rejection on autopsy.

Table 3 Outcome of eight patients suffering from chronic ductopenic rejection, in terms of response/ongoing rejection and survival/fatality, as a function of conversion to tacrolimus only or of conversion and additional OKT3 pretreatment

	Tacrolimus rescue + OKT3 ($n = 5$)	Tacrolimus rescue only ($n = 3$)
Response	3	2
Ongoing rejection	2	1
Survival	2	3
Fatality	3	0

Patients died almost exclusively of infectious complications. The most common final diagnoses were CMV and *Pneumocystis carinii* pneumonia in four cases each. In three patients, infections had been pre-existent: two cases of CMV pneumonia and one of CMV infection and *E.coli* sepsis. Additional causes of death observed in one patient each were aspergillosis, aggravated HCV reinfection, and lymphoma. The patient who developed post-transplant lymphoproliferative disease (PTLD) was among the first to receive tacrolimus in 1990; he had already undergone three cycles of OKT3 for treatment of chronic rejection before tacrolimus became available to us.

Comparing those patients who, for chronic ductopenic rejection, were converted to tacrolimus only with those who had, in addition, received at least one course of OKT3, a rather balanced pattern for rejection response was found (Table 3). None of the patients who died had solely been on tacrolimus; all had undergone a previous course of OKT3. However, neither correlation was statistically significant.

Graft loss unrelated to patient death was observed twice (Table 2). These two patients underwent successful retransplantation for chronic ductopenic rejection. Grafts were lost 4 and 5 months post-transplantation, or 2 and 3 months post-switch, respectively. In another patient with an ongoing VBDS, retransplantation had not been considered because of a manifest CMV pneumonia and an *E.coli* sepsis with a multiple organ failure syndrome. Tacrolimus rescue had been tried as a last resort; however, the patient died 2 months after the switch.

Conversion to tacrolimus was based on an intent to treat. Mainly during the early phase of our program, prior to our initial experience with tacrolimus or its availability, 15 patients received OKT3 alone for treatment of cellular steroid-resistant rejections. Ten patients

recovered (67 %), while one patient died and four underwent retransplantation for refractory rejection. An additional 13 patients had to be switched to tacrolimus for progressive rejection after OKT3 therapy.

Adverse events

Adverse events mainly included infection, renal insufficiency, or neurological disorder and were pre-existent in a total of 21 patients (64 %). Their incidence could be divided into three categories: those arising de novo, those that persisted, and those that improved post-switch (Table 4).

De novo infections were observed in 15 patients (45 %), nephrotoxic or neurotoxic effects in 11 (33 %) and 13 (39 %) cases, respectively. Persistence or aggravation of pre-existing infectious complications was evident in four patients (12 %), while that of pre-existing renal insufficiency or neurological disorders was seen in six (18 %) and three (9 %) cases, respectively. Improvement in prior disorders of an infectious nature occurred in nine patients (27 %); disorders of renal origin or related to a neurological site improved in five patients each (15 %).

De novo infection was mostly caused by opportunistic pathogens, i.e., CMV ($n = 4$), *Pneumocystis carinii* ($n = 4$), and *Legionella* ($n = 2$), as well as by fungi ($n = 5$). Diagnosis of de novo CMV ($n = 1$) or *Pneumocystis carinii* infection ($n = 4$) and of de novo aspergillosis ($n = 1$) was made in four of the eight lethal cases. Aggravation of pre-existing infection after conversion was mainly related to those caused by CMV ($n = 3$), and all of these took a lethal turn, in one patient suffering from chronic ductopenic, in one with OKT3-resistant cellular rejection, and in one with severe, steroid-resistant cellular rejection. Four pre-existing CMV infections improved post-switch in one patient each suffering from chronic ductopenic, early chronic, and severe, steroid-resistant cellular rejection, as well as in one patient belonging to the CyA malabsorption group. Bacterial cholangitis ($n = 5$) was the pre-existing complication that improved most often during successful rescue therapy irrespective of the secondary diagnosis.

An isolated rise in serum creatinine above 1.5 mg/dl or the need for hemodialysis as a complication after the onset of tacrolimus administration was evident in seven and four patients, respectively. Four patients undergoing hemodialysis had already done so prior to conversion. In another four patients, the post-switch kidney function improved to such an extent that hemodialysis was no longer necessary. Out of a total of 12 cases requiring hemodialysis, 7 had occurred in the group of steroid-resistant cellular rejections: in two rescue failures de novo, and in three and two successful cases persisting or improving, respectively. Another two patients who needed hemodialysis after conversion and for whom the rescue therapy failed belonged to the group of chronic ductopenic and OKT3-resistant rejection. In one successful rescue case in the chronic rejection group and another in the group of poor CyA uptake, pre-switch hemodialysis could be dispensed with after initial tacrolimus treatment. None of the nonlethal cases required hemodialysis for more than 8 weeks. However, renal insufficiency, as measured by a serum creatinine level between 1.6 and 3 mg/dl, was persistent in six patients during long-term follow-up.

Neurological disorders were further divided into minor and major disturbances, each observed in 11 (33 %) and 6 (18 %) patients, respectively. Since the clinical picture was compounded by minor and major manifestations, only 7 patients (21 %) presented solely with minor changes. The most prominent de novo neurological

Table 4 Incidence of adverse events in the study population listed as de novo, persistent, or improving complication related to the onset of tacrolimus therapy

Adverse events	De novo	Persistent	Improving
Infections			
CMV pneumonia	3	2	1
CMV (PCR positivity[a])	1	1	3
PCP	4		
Legionella pneumonia	2		
Fungal infections	5	1	2
Bacterial cholangitis	3		5
Urinary tract infection	2		2
Bacterial pneumonia	1		1
E. coli sepsis		1	
Total	21 in 15 patients	5 in 4 patients	14 in 9 patients
Renal insufficiency			
Serum creatinine > 1.5 mg/dl	7	2	1
Hemodialysis requirement	4	4	4
Total	11	6	5
Neurological disorders			
Minor Tremor	13	2	2
Mood changes	2		
Somnolence	2		
Headache	1		
Peripheral neural disorders	1	1	
Major Organic mental syndrome	3		1
Psychosis	2		
Seizures	1		
Encephalopathy			3
Personality disorder			1
Dysarthria			1
Ataxia			1
Total	25 in 13 patients	3 in 3 patients	9 in 5 patients

[a] Polymerase chain reaction *(PCR)*-directed detection of CMV envelope in blood

Table 5 Comparison of pre-switch laboratory values as a function of the indication for conversion to tacrolimus. Groups were divided according to outcome if failures were evident. Values represent mean ± SEM

	Bilirubin (mg/dl)	AP (IU/l)	γ-GT (IU/l)	AST (IU/l)	ALT (IU/l)
Chronic rejection					
Success	16.6 ± 11.0	727 ± 429**,***	640 ± 195***,**	88 ± 38	194 ± 83
Failure	13.7 ± 4.9	756 ± 419**,***	626 ± 470***,**	70 ± 47	91 ± 45
OKT3-resistant cellular rejection					
Success	16.3 ± 4.4	214 ± 81	318 ± 212	51 ± 31	131 ± 97
Failure	20.7 ± 2.3	341 ± 4	604 ± 336	89 ± 33	135 ± 8
Severe steroid-resistant cellular rejection					
Success	12.0 ± 5.6	245 ± 189	293 ± 149	48 ± 36	106 ± 35
Failure	29.7 ± 5.9*	338 ± 231	376 ± 350	37 ± 13	95 ± 9
Early chronic rejection	12.7 ± 6.2	310 ± 203	158 ± 127	55 ± 46	114 ± 69
Late onset cellular rejection	5.7 ± 5.3	339 ± 207	270 ± 182	108 ± 79	168 ± 99
Cellular rejection and CyA malabsorption	11.9 ± 7.9	516 ± 475	197 ± 139	103 ± 90	222 ± 196

* $P < 0.05$ compared with the respective successful rescue cases; ** $P < 0.05$ compared with the subset of the study population not suffering from chronic rejection; *** $P < 0.05$ compared with the early chronic rejection group

complication after conversion to tacrolimus was tremor ($n = 13$). It did not display a predilection for any of the secondary diagnoses and occurred in four of the eight lethal cases. However, neurological evaluation in the moribund and critically ill tended to be impeded by sedation or relaxation intended for optimal ventilatory support. Both cases of somnolence were diagnosed in patients who later died of CMV and *Pneumocystis carinii* pneumonia. Of the neurological disorders that improved during the rescue therapy, encephalopathy ($n = 3$) was most eminent. Its etiology was metabolic and occurred in one successful rescue case in the chronic ductopenic rejection group and in one in the steroid-resistant cellular rejection group, as well as in one patient suffering from cellular rejection in the CyA malabsorption group.

Laboratory findings

Pre-switch laboratory parameters in the groups where failures had occurred were checked for a putative predictive potency (Table 5). A significantly elevated total serum bilirubin in unsuccessful cases compared to successful ones (29.7 ± 5.9 mg/dl vs 12.0 ± 5.6 mg/dl; $P < 0.05$) was found in the group with steroid-resistant cellular rejection. Prior to conversion in the chronic ductopenic rejection group, total serum bilirubin levels were, in contrast, higher in patients who profited from the rescue therapy. Among OKT3 nonresponders, the levels of total serumbilirubin, as well as alkaline phosphatase (AP) and γ-glutamyltransferase (γ-GT) activity, were elevated slightly, though not significantly in those where rescue was about to fail. In the other groups, AP and γ-GT activity, as well as levels of the aminotransferases (AST, ALT), did not differ between rescue cases that failed and those that succeeded.

Intergroup analysis did not disclose any statistically significant differences, except for the chronic rejection group. In these patients, whether treatment failed or not, AP and γ-GT serum activity was significantly ($P < 0.05$) elevated in comparison to all those suffering from rejection other than chronic (743 ± 423 IU/l vs 305 ± 207 IU/l, and 630 ± 378 IU/l vs 267 ± 236 IU/l, respectively). Serum bilirubin, AST, and ALT levels did not display significant differences. Moreover, the only distinction found between early chronic and chronic ductopenic rejection was in a significantly differing AP (310 ± 203 IU/l vs 743 ± 423 IU/l; $P < 0.05$) and γ-GT serum activity (158 ± 127 IU/l vs 630 ± 378 IU/l; $P < 0.05$). Serum bilirubin levels were only slightly lower in the early chronic rejection group (12.7 ± 6.2 mg/dl vs 15.9 ± 8.5 mg/dl; P = NS).

Discussion

In 33 patients suffering from various kinds of liver allograft rejection, conversion from CyA-based immunosuppressive regimens to tacrolimus and steroids generated response and success rates of 88 % and 70 %, respectively. Outcome was mostly dependent on the entry diagnoses for tacrolimus rescue therapy. Complete response and fair overall outcome were characteristic for all patients suffering from early chronic rejection, late onset cellular rejection, and cellular rejection based on CyA malabsorption. While these figures correspond to those from previous reports [4, 8], patients with severe steroid-resistant or OKT3-resistant cellular rejection have not fared as well in our experience. Although response rates ranged high in both groups – 88 % and 100 %, respectively – only a limited reliability in terms of patient survival – about 65 % in each group – was ob-

served. These data tend to support studies that are less enthusiastic about an incontestable role of tacrolimus in cellular rejection [13, 26]. In manifest ductopenic rejection, a restricted benefit of tacrolimus rescue therapy is a well-known feature [4, 8, 13, 15, 36]. In our series, the response and graft survival figures were 63 % and 38 %, respectively.

Opportunistic infections, i.e., CMV and *Pneumocystis carinii* pneumonia, accounted for most of the rescue failures. Although in 50 % of all cases undergoing conversion to tacrolimus many infectious, renal, and neurological complications had already pre-existed, the *Pneumocystis carinii* pneumonias were acquired de novo. It might be worth noting that these patients had undergone transplantation prior to a change in our perioperative prophylaxis from aerosolized pentamidine to bactrim. All patients had received 10 g of immunoglobulin 7S and CMV hyperimmunoglobulin on postoperative days 1 and 14. While the incidence of infections was not elevated in primary protocols entailing tacrolimus, a rising rate was evident in the same study among those converted [12]. A distinct risk of conversion was also confirmed by another report mirroring our *Pneumocystis carinii* pneumonia incidence of 12 % [13].

Though not statistically significant, previous OKT3 therapy emerged as unfavorable in patients switched for chronic ductopenic rejection. In the group of OKT3-resistant cellular rejection, both patient deaths were due to CMV pneumonia, one of which had developed pre-switch. Therefore, tacrolimus rescue therapy should be considered very cautiously in patients with prior OKT3 treatment. Although tacrolimus rescue studies are subject to the pitfalls of compassionate use protocols, it might be advisable to avoid OKT3 therapy in general in favor of an early switch to tacrolimus. While the survival figures of 15 patients after treatment with OKT3 for steroid-resistant cellular rejection did not differ from those of patients converted to tacrolimus, a total of 13 additional patients initially treated with OKT3 alone had to undergo conversion for refractory rejection later on compared to only one patient with ongoing cellular rejection after a switch to tacrolimus.

Most patients were high-risk cases, as reflected in the 21 patients (64 %) presenting with pre-existing infectious, renal, or neurological disorders, and in the time interval between onset of tacrolimus conversion and patient death, which was sometimes as short as 1 week. In successfully converted patients, the benefit did not only apply to control of rejection but also to these pre-existing disorders. The general improvement might have been associated with a gain in liver function or with previous drug induced toxicity. Mainly bacterial infections, among them cholangitis, were affected by less impaired, nonspecific defenses and a normalization in bile flow [2, 9]. Kidney function was enhanced to such an extent that four patients requiring hemodialysis prior to conversion no longer needed it afterwards. Neurological disorders that were relieved after conversion were predominantly metabolic and, therefore, profiled of improvements in liver and kidney function.

Conversely, post-switch nephrotoxicity and neurotoxicity were experienced by 33 % and 36 % of the patients, respectively. Possible contributing factors such as infections or nephrotoxic antibiotics not even taken into account, the number of patients exhibiting renal dysfunction was considerably lower than the initial 80 %–90 % figures of previous rescue studies [10, 16]. This difference is most likely due to the relatively low dose of tacrolimus administered orally rather than intravenously. It is known that nephrotoxicity is dose-dependent and can be managed with subsequent adjustments [17]. In our hands, the monitoring of tacrolimus plasma levels, which had been evaluated in our 33 patients, clearly failed to detect tacrolimus overimmunosuppression and were, therefore, an unreliable parameter. Improved monitoring of whole blood instead of plasma levels, which are more reliable in detecting overimmunosuppression, and earlier dose reduction to a lower therapeutic range may further improve the outcome of tacrolimus-treated patients.

Neurotoxicity has been described as most common after intravenous tacrolimus administration; yet, even profound neurological events tend to be temporary in nature [6]. In our series, 19 % of cases demonstrated minor neurotoxicity, mostly noted as tremors of the hand. These results are comparable to those of intravenous tacrolimus use in primary immunosuppression [6]. Like nephrotoxicity, neurological dysfunction occurred significantly more often in rescue cases than in primarily treated patients [30]. The 17 % of major neurological disturbances identified among our patients exceeded the 5 % reported for primary and intravenous tacrolimus use. However, all de novo neurological complications resolved during follow-up.

In their review of 96 patients converted from CyA to tacrolimus, Demetris and coworkers found that in chronic ductopenic rejection the combination of a total serum bilirubin level exceeding 20 mg/dl and a greater than 50 % bile duct loss on biopsy was highly predictive of nonresponse [4]. A duct loss of 50 % or more was an inclusion criterion for the chronic rejection group in our study. Neither the bilirubin level nor the serum activity of hepatocellular or canalicular enzymes was predictive for outcome. The extent of bile duct loss served as the basis for distinguishing between early chronic and chronic ductopenic rejection, and was reflected in significantly elevated canalicular enzyme serum activity in the chronic rejection group. However, these parameters failed pre-switch to differentiate between success and failure. Not all patients rescued from chronic ductopenic rejection were biopsied during follow-up if the

clinical course was satisfactory. In those where normal graft function had resumed and a post-switch biopsy was obtained, histopathology was less impressive, if at all, than clinical outcome.

In cellular steroid-resistant rejection, the pre-switch total serum bilirubin was significantly elevated in unsuccessful cases compared to successful ones. In all patients from whom a biopsy was obtained after conversion, rejection had resolved completely. In contrast to chronic rejection, the rise in serum bilirubin was most likely due to impaired hepatocellular function without a significant role of duct loss. Since the predictive potency was confined to failure and not to nonresponse, it would appear that tacrolimus may be able to reduce a given extent of graft damage only at the expense of severe over-immunosuppression. This is all the more probable given the fact that patients in this group whose therapy failed died only of complications that had not pre-existed.

In conclusion, conversion to tacrolimus was a reliable treatment for early chronic rejection, late onset cellular rejection, and rejection based on CyA malabsorption. It was also potent in OKT3- and severe, steroid-resistant cellular rejection. However, tacrolimus rescue therapy was not beneficial to all of the patients in these latter groups and only in a subset of those suffering from chronic ductopenic rejection. In almost all failures, opportunistic infections represented the cause of death and, as in the U.S. multicenter trial, prior OKT3 treatment emerged as a risk factor [35]. The reverse was also true, namely, patients converted during an early stage of rejection profited the most. Benefits of conversion to tacrolimus did not only become evident with enhanced liver performance, but also with regard to pre-existing complications. Long-term toxicity was confined to mild renal insufficiency in six patients without a further need for hemodialysis. In severe, steroid-resistant cellular rejection, rescue failures presented a significantly elevated pre-switch total serum bilirubin compared to rescue successes. The underlying mechanisms of tacrolimus rescue in chronic rejection remain obscure, hence predictive parameters still need to be elucidated as well.

References

1. Blumhardt G, Bechstein WO, Steffen R, Keck H, Neuhaus P (1991) Experience with quadruple immunosuppression in 50 consecutive liver transplants. In: Engemann R, Hamelmann H (eds) Experimental and clinical liver transplantation. Excerpta Medica/Elsevier, Amsterdam, pp 219–227
2. Carey M (1982) The enterohepatic circulation. In: Arias I, Popper H, Schachter D, Shafritz DA (eds) The liver: biology and pathobiology. Raven Press, New York, pp 429–444
3. Cosimi AB, Cho SI, Delmonico SL, Kaplan MM, Rohrer RJ, Jenkins RL (1987) A randomized clinical trial comparing OKT3 and steroids for the treatment of hepatic allograft rejection. Transplantation 43: 91–95
4. Demetris AJ, Fung JJ, Todo S, McCauley J, Jain A, Takaya S, Alessiani M, Abu-Elmagd K, VanThiel DM, Starzl TE (1992) Conversion of liver allograft recipients from cyclosporine to FK506 immunosuppressive therapy – a clinicopathologic study of 96 patients. Transplantation 53: 1056–1062
5. Egawa H, Esquivel CO, So SK, Cox K, Concepcion W, Lawrence L (1994) FK506 conversion therapy in pediatric liver transplantation. Transplantation 57: 1169–1173
6. Eidelman BH, Abu-Elmagd K, Wilson J, Fung JJ, Alessiani M, Jain A, Takaya S, Todo S, Tzakis A, Van Thiel D, Shannon W, Starzl TE (1991) Neurologic complications of FK506. Transplant Proc 23: 3175–3178
7. Fung JJ, Todo S, Jain A, McCauley J, Alessiani M, Scotti C, Starzl TE (1990) Conversion from cyclosporine to FK506 in liver allograft recipients with cyclosporine-related complications.Transplant Proc 22: 6–12
8. Fung JJ, Todo S, Tzakis A, Alessiani M, Abu-Elmagd K, Jain A, Bronster O, Martin M, Gordon R, Starzl TE (1991) Current status of FK506 in liver transplantation. Transplant Proc 23: 1902–1905
9. Gluckman SJ, Dvorak VC, MacGregor RR (1977) Host defences during prolonged alcohol consumption in a controlled environment. Arch Intern Med 137: 1539–1545
10. Hebert MF, Ascher NL, Lake JR, Roberts JP (1991) Efficacy and toxicity of FK506 for the treatment of resistant rejection in liver transplant patients. Transplant Proc 23: 3109–3110
11. Kalayoglu M, Stratta RJ, Hoffmann RM, Sollinger HW, Belzer FO (1988) Quadruple immunosuppressive therapy for liver transplantation. Transplant Proc 20 [Suppl 1]: 524–529
12. Kusne S, Fung J, Alessiani M, Martin M, Torre-Cisneros J, Irish W, Ondick L, Jain A, Abu-Elmagd K, Takaya S, Todo S, Starzl TE (1992) Infections during a randomized trial compaing cyclosporine to FK506 immunosuppression in liver transplantation. Transplant Proc 24: 429–430
13. Lewis WD, Jenkins RL, Burke PA, Winn KM, Shaffer D, Lopez R, Monaco AP (1991) FK506 rescue therapy in liver transplant recipients with drug-resistant rejection. Transplant Proc 23: 2989–2991
14. Ludwig J (1992) Terminology of hepatic allograft rejection. Semin Liver Dis 12: 89–92
15. Makowka L, Svanas G, Esquivel C (1986) Effect of cyclosporin on hepatic regeneration. Surg Forum 37: 352–357
16. McCauley J, Fung JJ, Brown H, Deballi P, Jain A, Todo S, Starzl TE (1991) Renal function after conversion from cyclosporine to FK506 in liver transplant patients. Transplant Proc 23: 3148–3149
17. McCauley J, Fung JJ, Todo S, Jain A, Deballi P, Starzl TE (1991) Changes in renal function after liver transplantation under FK506. Transplant Proc 23: 3143–3145

18. Mor E, Solomon H, Gibbs JF, Holman MJ, Goldstein RM, Husberg BS, Gonwa TA, Klintmalm GB (1992) Acute cellular rejection following liver transplantation: clinical pathologic features and effect on outcome. Sem in Liver Dis 12: 28–40
19. Neuhaus P, Blumhardt G (1994) Extended bile duct resection – a new oncological approach to the treatment of central bile duct carcinomas? Description of method and early results. Langenbecks Arch Chir 379: 123–128
20. Neuhaus P, Bechstein WO, Blumhardt G, Wiens M, Lemmens P, Langrehr JM, Lohmann R, Steffen R, Schlag H, Slama KJ, Lobeck H (1993) Comparison of quadruple immunosuppression after liver transplantation with ATG or IL-2 receptor antibody. Transplantation 55: 1320–1327
21. Neuhaus P, Blumhardt G, Bechstein WO, Steffen R, Platz KP, Keck H (1994) Technique and results of biliary reconstruction using side-to-side choledochocholedochostomy in 300 orthotopic liver transplants. Ann Surg 219: 426–434
22. Noack KB, Wiesner RH, Batts, K, Hoek B van, Ludwig J (1991) Severe ductopenic rejection with features of vanishing bile duct syndrome: clinical, biochemical, and histologic evidence for spontaneous resolution. Transplant Proc 23: 1448–1451
23. Reding R, Vraux H, Ville de Goyet J de, Hemptinne B de, Latinne D, Rahier J, Jamart J, Vincenzotto C, Cormont F, Parra B de la, Bruyere M de, Sokal G, Bazin H, Otte JB (1993) Monoclonal antibodies in prophylactic immunosuppression after liver transplantation. Transplantation 55: 534–541
24. Reding R, Wallemacq PE, Lamy ME, Rahier J, Sempoux C, Debande B, Jamart J, Barker A, Sokal E, Ville de Goyet J de, Moulin D, Clement de Clety S, Otte JB (1994) Conversion from cyclosporine to FK506 for salvage of immunocompromised pediatric liver allografts. Transplantation 57: 93–100
25. Renard TH, Andrews WS, Foster ME (1991) Relationship between OKT3 administration, EBV seroconversion and the lymphoproliferative syndrome in paediatric liver transplantation. Transplant Proc 23: 1473–1476
26. Rucay P, Samuel D, Gillet D, Bismuth H (1991) FK506 rescue therapy for refractory acute rejection in five liver recipients. Transplant Proc 23: 3000–3001
27. Samuel D, Gugenheim J, Canon C, Saliba F, Bismuth H (1990) Use of OKT3 for late acute rejection in liver transplantation. Transplant Proc 22: 1767–1768
28. Schattenfroh N, Lange D, Bechstein WO, Blumhardt G, Keck H, Langrehr JM, Lohmann R, Neuhaus P (1993) Induction therapy with anti-T-lymphocyte globulin following orthotopic liver transplantation. Transplant Proc 25: 2702
29. Schmidt CA, Oettle H, Neuhaus P, Wiens M, Timm H, Wilborn F, Siegert W (1993) Demonstration of cytomegalovirus by polymerase chain reaction after liver transplantation. Transplantation 56: 872
30. Shapiro R, Fung JJ, Jain AB, Parks P, Todo S, Starzl TE (1991) The side effects of FK506 in humans. Transplant Proc 22 [Suppl 1]: 35–36
31. Shaw BW Jr, Markin R, Stratta R, Langnas A, Donovan J, Sorrell M (1991) FK506 for rescue treatment of acute and chronic rejection in liver allograft recipients. Transplant Proc 23: 2994–2995
32. Singh N, Dummer JS, Kusne S, Breinig MK, Armstrong JA, Makowka L, Starzl TE, Ho M (1988) Infection with cytomegalovirus and other herpesvirus in 121 liver transplant recipients: transmission by donated organs and the effect of OKT3 antibodies. J Infect Dis 158: 124–131
33. Starzl TE, Todo S, Fung J, Demetris AJ, Venkataramman R, Jain A (1989) FK506 for liver, kidney, and pancreas transplantation. Lancet II: 1000–1004
34. Thistlethwaite RJ, Stuart JK, Mayes JT, Gaber AO, Woodle S, Buckingham MR, Stuart FP (1988) Monitoring and complications of monoclonal therapy: complications and monitoring of OKT3 therapy. Am J Kidney Dis 11: 112–119
35. U.S. Multicenter FK506 Liver Study Group (1993) Prognostic factors for successful conversion from cyclosporine to FK506-based immunosuppressive therapy for refractory rejection after liver transplantation. Transplant Proc 25: 641–643
36. Winkler M, Ringe B, Gerstenkorn C, Rodeck B, Gubernatis G, Wonigeit K, Pichlmayr R (1991) Use of FK506 for treatment of chronic rejection after liver transplantation. Transplant Proc 23: 2984–2986