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ORIGINAL ARTICLE

Induction Immunosuppression With Rabbit Antithymocyte Globulin in Pediatric Liver Transplantation

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Routine use of rabbit antithymocyte globulin (RATG) induction therapy remains controversial in pediatric liver transplantation. We reviewed our experience of 18 cadaveric liver transplants in 18 children over a span of 2 years. All patients received the same immunosuppression: perioperative steroid therapy with taper, 3 doses of RATG, and maintenance therapy of steroids and tacrolimus started on postoperative day 3. Mean follow-up was 2.2 ± 0.2 years. End-stage liver disease was secondary to biliary atresia in 10 patients (56%) and metabolic disorders in 4 patients (22%). Graft and patient survival were 89%. Serum bilirubin was 1.2 mg/dL, 1.1 mg/dL, 0.5 mg/dL, and 0.5 mg/dL at 1, 3, 6, and 12 months, respectively. The 2 mortalities were secondary to multiple organ system failure. Overall rejection rate was 17% (3/18). Rejection episodes occurred at 4, 6, and 7 months. Two patients were treated with steroids; the third was treated with OKT3. No patient has developed posttransplant lymphoproliferative disease. Serum creatinine was 0.7 mg/dL, 0.6 mg/dL, 0.6 mg/dL, and 0.6 mg/dL at 1, 3, 6, and 12 months, respectively, among surviving patients. In conclusion, our data suggest that RATG induction with steroid and tacrolimus maintenance therapy is safe, easy to use, and effective in the prevention of rejection. ***Liver Transpl 12:1210-1214, 2006.***

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Routine use of rabbit antithymocyte globulin (RATG) induction therapy in liver transplantation remains controversial. Though induction therapy with RATG has been successful in adult liver transplantation[1] when used in conjunction with triple immunosuppression and steroid-free protocols, elevated incidences of cytomegalovirus (CMV) and posttransplant lymphoproliferative disease (PTLD) following the use of biologic agents for the treatment acute cellular rejection has dampened enthusiasm for these agents in induction protocols.[2] RATG is used as induction immunosuppression in only 11% of pediatric liver transplant recipients registered in the Studies of Pediatric Liver Transplantation (SPLIT) per the 2003 report.[3] In spite of its potency, fears that patients will have increased incidence of Epstein-Barr virus (EBV), CMV, and PTLD have limited the application of RATG in the pediatric liver transplantation population.

In addition to its potency as an induction agent, RATG allows for the delayed introduction of calcineurin inhibitor–based maintenance immunosuppression. The delayed introduction of calcineurin inhibition has several potential benefits to the recipient. Primarily, the use of calcineurin inhibitors can be avoided in the immediate postoperative period. Given the renal and neurological side effects associated with elevated serum trough levels, delaying the use of calcineurin inhibitors allows the clinician to evaluate the fresh postoperative liver transplant recipient in the absence of confounding variables. In addition, delayed introduction of maintenance immunosuppression allows for some recovery of renal function postoperatively prior to the addition of a nephrotoxic agent.

The most difficult time period in the management of the liver transplant recipient is in the first postoperative month. In the absence of confounding variables, eleva-

Abbreviations: RATG, rabbit antithymocyte globulin; CMV, cytomegalovirus; PTLD, posttransplant lymphoproliferative disease; SPLIT, Studies of Pediatric Liver Transplantation; EBV, Epstein-Barr virus.

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TABLE 1. Demographic Data in Pediatric Liver Transplant Recipients Between July 2001 and May 2003

Demographics	Median	Mean	Range
Age	3 y	7 y	5 mo to 14 y
Weight (kg)	16.5	27	6 to 68
PELD (n = 13)	2	3	−10 to 15
		Number	
Status 1		1	
Status 2B		3	
Status 3		1	
Sex			
Female		8	
Male		10	
EBV Stus			
Negative		10	
Positive		8	

Abbreviation: PELD, pediatric end-stage liver disease.

tions in liver chemistries may be more clearly investigated and classified as a result of significant reperfusion injury, vascular complications, biliary complications or rejection. An ideal immunosuppressive protocol would ease the management burden of the liver transplant recipient while maintaining a very low incidence of rejection (especially in the first 8 weeks post transplant), minimal infectious complications and minimal impact on other organ systems.

The results described in this paper summarize our experience with an immunosuppression protocol that utilizes maintenance steroids given after graft reperfusion, 3 doses of RATG on initiated on postoperative days 1-3, and tacrolimus started on postoperative day 3 or when serum creatinine had normalized. Our results suggest that this protocol is safe, easy to use, and associated with a low incidence of rejection and few serious infectious risks.

PATIENTS AND METHODS

Patient Population

Between July 2001 and May 2003, 18 liver transplants were performed at Riley Children's Hospital with the intention of immunosuppression with RATG, tacrolimus, and steroids. Demographic data is shown in Table 1. Eighteen patients received 18 whole-organ liver transplants and were included in the RATG, tacrolimus, and steroid protocol. The disease breakdown is shown in Table 2.

Immunosuppression

Induction immunosuppression consisted of 7 mg/kg of methylprednisolone given intravenously following graft reperfusion or in the intensive care unit immediately following surgery. A standard steroid taper was used in all patients so that by one week posttransplant all patients were on 1 mg/kg of prednisone daily. Patients were weaned further to 0.25 mg/kg daily by 3 weeks. Three doses of RATG (2 mg/kg/dose) were given on alternating days starting on postoperative days 1-4. This schedule was extended in patients with renal insufficiency until creatinine had normalized. The timing of the first dose was dependent upon the patient situation. RATG was delayed if the patient was being weaned to extubation on the first postoperative day or if the patient was hemodynamically unstable. Tacrolimus was usually initiated on postoperative day 3, or when serum creatinine had normalized, and given to achieve 12-hour trough levels between 6 and 10 ng/mL.

Infectious Prophylaxis

All patients received 3 months of valgancyclovir for CMV and EBV prophylaxis. Valgancyclovir was discontinued after 3 months or if not tolerated. Therapy was restarted and combined with Cytogam (MedImmune, Gaithersburg, MD) if there was evidence of EBV infection. All patients received Bactrim (Roche, Nutley, NJ) for *Pneumocystis carinii* pneumonia prophylaxis.

Work-up of Elevated Liver Tests and Diagnosis of Rejection in the First 8 Weeks Posttransplant

In the immediate postoperative period elevations in transaminases were evaluated with Doppler ultrasound to exclude vascular complications. If Doppler ultrasound failed to reveal the cause of elevations in transaminases, then patients were evaluated by endoscopic retrograde cholangiopancreatography for signs of biliary obstruction or leak in patients with duct-to-duct anastomosis. A transhepatic cholangiogram was obtained for patients that had a choledochojejunostomy. Elevations in the cholestatic markers (total bilirubin, alkaline phosphatase, and gamma-glutamyltransferase) were evaluated by endoscopic retrograde cholangiopancreatography or transhepatic cholangiogram. If biliary pathology was present during endoscopic retrograde cholangiopancreatography or transhepatic cholangiogram, then a biliary stent was placed across the bile duct anastomosis. If Doppler ultrasound and endoscopic retrograde cholangiopancreatography

TABLE 2. Cause of End-stage Liver Disease in Pediatric Liver Transplant Recipients Between July 2001 and May 2003

Disease Process	Number
Biliary Atresia	10
A1A deficiency	2
Wilson's disease	1
Primary sclerosing cholangitis	1
Congenital hepatic fibrosis	1
Ornithine transcarbamylase deficiency	1
Hepatitis C recurrence	1
Subfulminant liver failure	1
Total	18

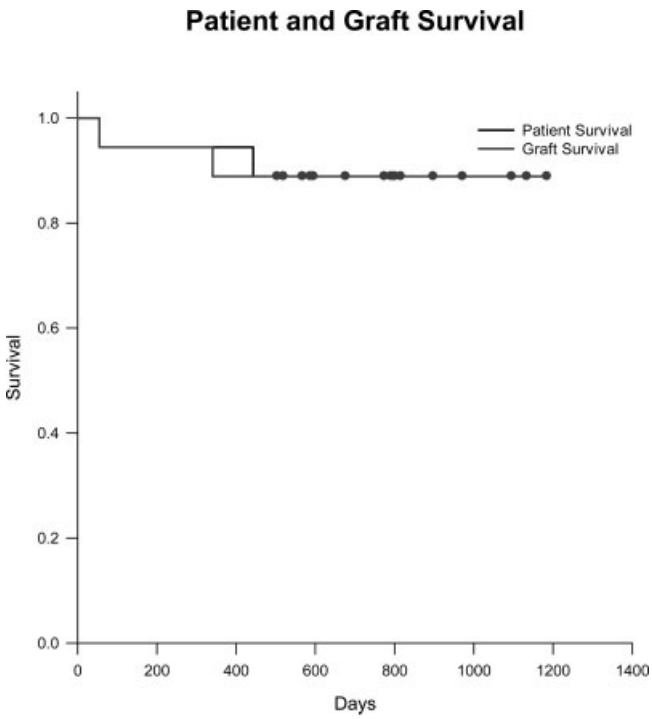

Figure 1. Actuarial patient (thick line) and graft (thin line) survival.

failed to reveal a cause for any enzyme elevations, a percutaneous liver biopsy was performed. Liver biopsies were evaluated by a pathologist in conjunction with a transplant surgeon and graded for rejection using the Banff criteria for allograft rejection. Patients who had rejection confirmed by biopsy were pulsed with steroids.

RESULTS

Patient and Graft Survival

Patient survival was 89% (16/18) and graft survival was 89% (16/18) (Fig. 1). One child was intubated on continuous veno-venous hemodialysis (CVVHD) pretransplant; she died of multiple organ system failure during the postoperative period. Two (11%) children had hepatic artery thrombosis and were subsequently revascularized. One child had no long-term sequelae. The other had biliary strictures requiring biliary stents. Three additional patients developed biliary stenosis. Two required biliary stents, while the third underwent revision of the choledochojejunostomy. One child had portal vein thrombosis and biliary strictures and underwent retransplantation. She died of multiple organ system failure during the postoperative period.

Incidence of Rejection and Its Treatment

Thirteen patients underwent liver biopsy during their posttransplant care. No patient had biopsy confirmed rejection within the first 2 months posttransplant. One patient had elevation of transaminases at 6 months that was treated with an increase in steroid dose and a recycle. Another patient had an undetectable tacrolimus level for 1 week and came back with serious transaminase elevations at 4 months. Biopsy revealed severe rejection treated with OKT3. A third patient's immunosuppression was held secondary to recurrent viral infections. The patient demonstrated elevation of liver enzymes at 8 months. Biopsy at that time revealed moderate rejection. Previous immunosuppression was resumed and subsequent biopsy was negative for rejection. All 3 patients have done well since their treatment for rejection.

Incidence of PTLD and Other Opportunistic Infections

No patient has developed PTLD. One patient had EBV detectable by polymerase chain reaction (PCR) posttransplant. That patient has been maintained on Valgancyclovir, and his viral load is currently undetectable. One patient had liver biopsy–confirmed adenovirus that was treated by decreasing immunosuppression. This patient also suffered from recurrent RSV. Immunosuppression was held temporarily. It has been resumed and the patient's symptoms have resolved.

Postoperative Renal Function

One patient was on continuous venovenous hemodialysis preoperatively and postoperatively. She never regained renal function. No other patient required preoperative or postoperative dialysis. Excluding this patient, mean creatinine was 0.7 mg/dL, 0.6 mg/dL, 0.6 mg/dL, and 0.6 mg/dL at 1, 3, 6, and 12 months, respectively (Fig. 2).

DISCUSSION

Our series of pediatric transplantation has demonstrated good survival without severe complications. Patient and graft survival has been 89% at 1 year; SPLIT has shown 1-year patient and graft survival of 87% and 82%, respectively. There were 3 vascular complications: 2 patients had hepatic artery thrombosis and 1 patient developed portal vein thrombosis. Of these only 1 required subsequent retransplantation. SPLIT data demonstrate the rate of vascular complications of 15%. Twenty percent of these patients suffered graft loss.[3]

Only 3 (17%) patients experienced rejection. All episodes occurred after the first 2 months posttransplant. Two of these episodes were related to low tacrolimus levels (<3.0 ng/mL). SPLIT reports rejection rate over 40% with a median time to first episode of 17 days. Using induction therapy we have achieved a significantly lower rate of rejection. One patient required OKT3 treatment for rejection. Within the SPLIT study,

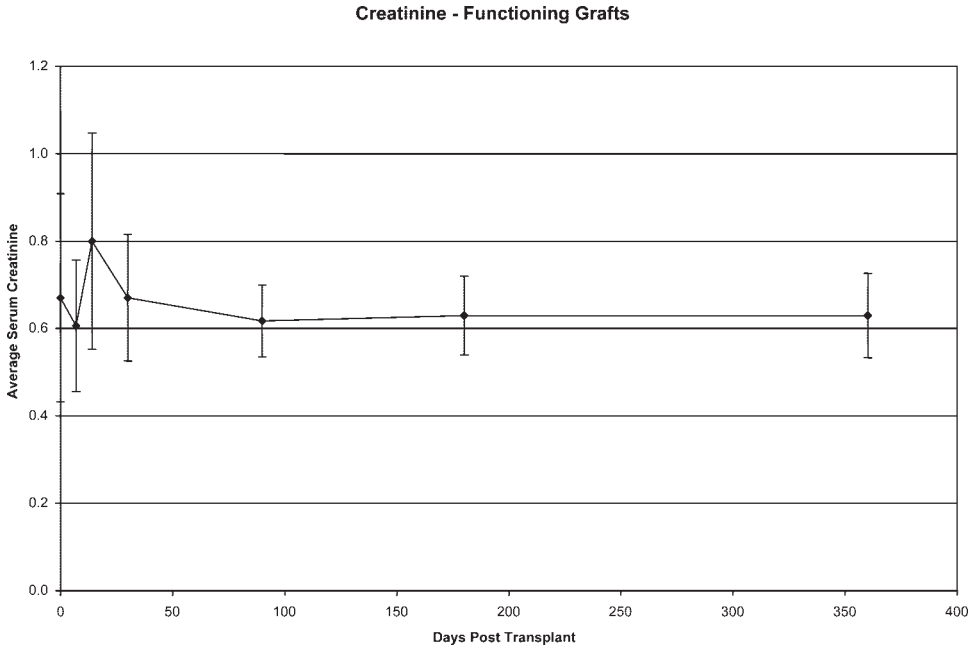

Figure 2. Serum creatinine in patients with functioning grafts.

5% of patients required treatment with biologic agents for steroid resistant rejection.[3]

The other major concern with the use of biologic induction therapy is that of an increased rate of PTLD or CMV. To date, no patient in our series has developed PTLD. Our median follow-up was 26 months; this is well below published rates between 6 and 11% for this time period. While 8 patients were EBV positive by serology prior to transplantation, only 1 patient developed EBV infection posttransplant. No patients have had evidence of CMV infection. No viral infection resulted in serious sequelae.

Finally, in spite of having a low rate of rejection within this series, our immunosuppressive protocol has demonstrated no evidence of renal toxicity in patients with a surviving graft. Liver transplantation has been associated with an increased risk of the development of end-stage renal disease.[4] Additionally, Alonso indicates that creatinine levels 1-year posttransplant are predictive of long-term renal function. Excluding the 1 child who developed multisystem organ failure and was initially on CVVHD prior to transplantation, no patient has progressed to end-stage renal disease. In addition, there has been no statistical increase in serum creatinine in these patients.

Similar data have been published regarding the use of OKT3 in both liver and multivisceral transplantation. McDiarmid et al.[5] published a series of 52 patients randomized to cyclosporine of OKT3 induction therapy after liver transplantation. They also showed a reduction in early rejection with OKT3 induction. Interestingly, they showed a decrease in infectious complications in patients with OKT3 induction as compared to those who required OKT3 rescue therapy after a rejection episode. They showed no difference between the OKT3 and cyclosporine groups in terms of renal function, though it is important to note that those patients on cyclosporine induction therapy developing renal insufficiency post transplant were switched to the OKT3 induction therapy. Some of these patients required hemodialysis.

In multivisceral transplantation, induction therapy has also been used extensively. Tzakis et al.[6] reviewed their experience of 100 multivisceral transplants. They were able to demonstrate a decrease in early rejection with induction therapy. In addition, they were also to show the maintenance of glomerular filtration rate in spite of long-term calcineurin inhibitor therapy in children. Interestingly, in multivariate analysis, they demonstrated the use of Campath-1H as an independent risk factor for patient death in children. While this is not attributable to early rejection, there also appears to be no increase in infectious complications in these patients.

Our immunosuppressive protocol with RATG induction and long-term tacrolimus monotherapy has been shown to be safe and effective. Similar to other series, we have demonstrated good patient and graft survival with significantly lower rates of rejection as compared to historical controls. Importantly, these lower rates of rejection have not come at the expense of elevated rates of opportunistic infections, EBV, CMV, or PTLD. In addition, we have demonstrated the use of tacrolimus without apparent renal toxicity.

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