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Clinical use of tacrolimus (FK-506) in infants and children with renal transplants

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Abstract. Although cyclosporine (CsA)-based immunosuppressive regimens have been highly successful in renal transplantation in infants and children, their adverse influence on somatic growth, general appearance, and blood pressure are of particular importance in this population. Over the past 4 years, we have utilized tacrolimus (formerly FK-506) as the primary immunosuppressive agent in 43 unselected children and achieved 1-year and 3-year allograft survival rates of 96% and 85%, respectively. We have also used tacrolimus to rescue 14 of 19 (74%) renal allografts from CsA-resistant rejection. Corticosteroids were discontinued in 62% of non-rescue patients without increasing the risk of rejection or renal dysfunction over a mean follow-up time of 25 months. Tacrolimus monotherapy has been associated with improved body growth and less obesity, while tacrolimus alone or in combination with prednisone was virtually free of hirsutism or gingival hypertrophy, and posed a low risk for hypertension. A major disadvantage of this regimen may be an increased risk for viral infections and a benign form of posttransplant lymphoproliferative disease. This article describes the tacrolimus protocol utilized in our center and focuses on practical clinical issues including therapeutic monitoring, benefits, and major toxicity in children with renal allografts.

Key words: Renal transplantation – Tacrolimus, FK-506 – Immunosuppression

Pharmacology and actions

Tacrolimus, formerly known as FK-506, is a polycyclic macrolide produced by a strain of *Streptomyces tsukubaensis* [1]. Intestinal absorption of tacrolimus is less dependent on bile acids than cyclosporine A (CsA). It is metabolized almost exclusively by the liver and is excreted in bile. The molecular basis for the immunosuppressive action of tacrolimus has been described [2, 3]. The interaction of the T-cell receptor with major histocompatibility complex-bound antigens or other T-cell activating factors leads to the activation of phospholipase C, which generates free intracellular calcium (Ca^{2+}). The Ca^{2+} binds to calmodulin, which in turn binds to and activates calcineurin, a serine/threonine phosphatase. This is a key enzyme in the eventual transcription of interleukin-2 and other lymphokines involved in the activation and proliferation of T-cells. Tacrolimus binds tightly to a specific 12-kilodalton cytosolic FK-binding protein (FKBP12) and less tightly to other isoforms of this distinct immunophilin family of proteins. The ensuing FKBP12-FK-506 complex binds to and neutralizes the phosphatase activity of the Ca^{2+}-calmodulin-calcineurin complex, thereby inhibiting the Ca^{2+}-dependent pathway of T-cell activation. CsA binds to its own receptor proteins or cyclophilins to form complexes, which also inhibit calcineurin activation. The greater potency of tacrolimus compared with CsA may be due to greater cell permeation and greater affinity for FKBP12. The relatively specific action of CsA and tacrolimus on T-lymphocytes, in contrast to non-selective agents such as azathioprine and corticosteroids, is incompletely understood. This action may relate to cell-specific isomers and a lower concentration of calcineurin or calcineurin substrates in T-lymphocytes, which may render these cells more susceptible to inhibition by immunophilin-ligand complexes.

On an equimolar basis, tacrolimus is 10 to 100 times more potent than CsA, and tacrolimus has not only been shown to prevent rejection, but also to reverse ongoing rejection in animals and humans [4, 5]. This latter property has enabled the conversion of individuals with CsA-resistant rejections to tacrolimus, resulting in the "rescue" of a substantial number of renal allografts.

Immunosuppression

Tacrolimus regimen

The basic tacrolimus protocol utilized at our center is outlined in Table 1. This protocol was modified from an

Table 1. Tacrolimus immunosuppressive protocol

Preoperative	Tacrolimus 0.15 mg/kg p.o.
Induction	Tacrolimus 0.075–0.1 mg/kg per day as a continuous infusion postoperatively Methylprednisolone 15 mg/kg IV (1 g maximum) during the operation
Maintenance	Tacrolimus 0.1 mg/kg per day IV with gradual tapering after starting oral intake at 0.15 mg/kg twice daily Prednisone or prednisolone 3 mg/kg daily on postoperative day 1 tapered to 0.75 mg/kg daily by postoperative day 6 Prednisone 0.25–0.5 mg/kg per day by 1 month with subsequent tapering ± azathioprine 2–3 mg/kg per day selectively if there is recurrent rejection
Tacrolimus whole blood levels	15–25 ng/ml during IV infusion 10–15 ng/ml in the 1st month 5–10 ng/ml after the 1st month

Table 2. Treatment of rejection

Steroids	
A. Methylprednisolone	15 mg/kg (1 g maximum) IV on day once, then 0.75 mg/kg IV every 6 h × 4 doses, then 0.60 mg/kg IV every 6 h × 4 doses, then 0.45 mg/kg IV every 6 h × 4 doses, then 0.30 mg/kg IV every 6 h × 4 doses , then 0.30 mg/kg IV every 12 h × 2 doses, then
Prednisone	0.30 mg/kg orally once daily
B. Methylprednisolone	15 mg/kg (1 g maximum) IV daily for 3 days, then
Prednisone	0.30 mg/kg p.o. once daily
C. Methylprednisolone	(or oral prednisone) 2.0 mg/kg (80 mg maximum) in 4 divided doses × 2 days, then 1.75 mg/kg in 4 divided doses × 3 days, then 1.50 mg/kg in 4 divided doses × 3 days, then 1.25 mg/kg in 4 divided doses × 3 days, then 1.0 mg/kg in 4 divided doses × 3 days, then 0.75 mg/kg in 2 divided doses × 3 days, then 0.5 mg/kg in 1–2 divided doses × 3 days
Antilymphocyte preparations	
A. OKT3	5 mg per day IV × 10–14 days (2.5 mg per day for weight <20 kg)
B. ALG	10–15 mg/kg per day IV × 14 days

ALG, Antilymphocyte globulin

earlier version [6] and reflects our increased experience with the agent. A small preoperative oral dose of tacrolimus is given within 5 h of surgery, and a continuous intravenous infusion is begun in the intensive care unit immediately upon completion of transplantation. Once the child is able to tolerate feedings, tacrolimus is given orally at a daily dosage 3 to 4 times higher than the intravenous dose. Because early graft dysfunction has not been an important complication in our patients, antilymphocyte preparations are not used. However, if there is no immediate renal function intraoperatively, it may be advisable to utilize antilymphocyte globulin for induction and to delay the start of tacrolimus until renal function is established.

The objective of the intravenous tacrolimus dosing is to achieve whole blood concentrations between 15 and 25 ng/ml. The objective of oral dosing is to maintain 12-h trough whole blood levels ranging from 10 to 15 ng/ml during the 1st postoperative month and subsequent therapeutic whole blood levels between 5 and 10 ng/ml. These levels have been associated with minimal toxicity. Target levels are usually met by administering tacrolimus twice per day 1 h before or 2 h after a meal or antacid administration. Ten percent of our patients received either one or three doses per day. These low or high dosages of tacrolimus were utilized to maintain therapeutic drug levels. After a mean follow-up time of 25 months, the daily tacrolimus dosage for the entire group was 0.19 ± 0.15 mg/kg (mean ± SD).

Intravenous methylprednisolone is begun at a dosage of 15 mg/kg (not to exceed 1 g) in the intraoperative period. This is followed by a prednisolone or prednisone taper from 3 mg/kg daily on postoperative day 1 to 0.75 mg/kg per day by postoperative day 6. By 1 month after transplantation, the majority of children received a daily prednisone dosage between 0.25 and 0.50 mg/kg. In children without rejection or with easily reversible rejection, the prednisone dosage is gradually tapered starting at 1 month after transplantation, and discontinued between 3 and 6 months. Steroid tapering and withdrawal are slowed (7 and 12 months) in children with a panel-reactive antibody level over 40%, a prior allograft loss due to recurrent glomerulonephritis, a history of recurrent rejection during previous transplantation, or an uncomplicated course after tacrolimus rescue therapy.

Additional medications are routinely utilized to prevent or decrease the severity of potential complications linked to immunosuppressive therapy or viral transmission by the allograft. Famotidine (Pepcid 0.3 mg/kg once at bedtime) and sucralfate (Carafate 250–500 mg 4 times daily before meals) are given to prevent or reduce gastrointestinal symptoms from high-dose steroids during the first 3 weeks and a calcium carbonate preparation (Tums) may be given with the prednisone. In cytomegalovirus (CMV)-seronegative recipients of CMV-seropositive renal donors, hyperimmune CMV immunoglobulin (Cytogam) is initially infused at 150 mg/kg with biweekly dosages over 4 months. Other prophylactic agents include acyclovir (650 mg/m^2 per day) for 6 months, co-trimoxazole (4 times daily for at least 1 year), and mycostatin (3 times daily for 2 months).

Acute rejection

Although 58% of our 24 initial patients experienced an early biopsy-confirmed acute rejection [6], the incidence fell below 40% under the modified immunosuppression protocol (Table 1). Because acute nephrotoxicity may occasionally occur at therapeutic blood levels of tacrolimus, and because urinary findings and ultrasound and renal scan studies are often non-specific, an allograft biopsy is recommended before starting antirejection therapy. Rejections are typically mild, and may be easily controlled by increasing the tacrolimus dosage and by using one of the

Table 3. Drug interactions of tacrolimus

Medications which may increase tacrolimus levels		Medications which may decrease tacrolimus levels
Erythromycin	Verapamil	Phenytoin
Methylprednisolone	Cimetidine	Phenobarbital
Clotrimazole	Itraconazole	Rifampin
Fluconazole	Danazol	Carbamazepine
Ketoconazole	Diltiazem	Cyclosporine

three steroid regimens shown in Table 2. Regimens A and B are reserved for more serious rejections and regimen C for milder grades, according to the histological guidelines employed at our center [7]. Only 3 of our patients were given antilymphocyte globulin to overcome early rejection. These 3 and 2 other children with rejection were then started on azathioprine at a dosage of 2–3 mg/kg per day.

Rescue

Children with recurrent biopsy-confirmed allograft rejections with high CsA therapeutic blood levels who fail treatments with high-dose steroids plus antilymphocyte preparations (antilymphocyte globulin or OKT3), and who have had a recent biopsy demonstrating a significant potential for recovery, may be candidates for conversion to tacrolimus or rescue therapy. Typically, CsA is stopped and patients are continued on a basal oral steroid dose and on their usual azathioprine dose, while tacrolimus is given orally at a dosage of 0.15 mg/kg twice daily. Provided that the response is favorable, azathioprine is discontinued and steroids are reduced or discontinued.

Precautions

Tacrolimus has a relatively narrow therapeutic range; therefore, drug interactions and other factors which may alter its levels in the blood must be carefully monitored. A growing number of medications are known to inhibit hepatic microsomal metabolism, and thereby increase tacrolimus levels in the blood and the potential for drug toxicity (Table 3). More importantly, some agents may stimulate microsomal metabolism resulting in reduced blood levels and an increased risk of rejection, particularly when tacrolimus is utilized as monotherapy. Concomitant use of nephrotoxic drugs may potentiate the renal toxicity of tacrolimus. Therefore, whenever possible, non-steroidal antiinflammatory agents should be avoided. When amphotericin B is utilized, its dosage should be decreased or given on alternate days. Until further data become available, precautions similar to those reported with CsA and its drug interactions may also be applied to tacrolimus.

Nephrotoxicity due to tacrolimus may be exacerbated by diarrhea, vomiting, or diuretic-induced hypovolemia. Therefore, adequate hydration should be stressed. Because diarrhea appears to have less of an influence on tacrolimus than CsA absorption, dosage adjustments are usually not needed. On occasion, however, tacrolimus blood levels may become unpredictable, particularly in infants with diarrhea or short gut syndrome. Non-infectious diarrhea has been reported in adults receiving tacrolimus [8] and was seen in 3 of 7 children between 2 and 5 years of age in our own series. Two of these children responded to a brief course of ioperamide hydrochloride (Imodium). In the third child with a more protracted course, tacrolimus was given intravenously for several days to successfully disrupt the diarrhea cycle, while reducing the risk of rejection.

Other factors which may reduce blood levels of tacrolimus include: (1) intake with meals, (2) the concomitant infusion or oral administration of antacids or sodium bicarbonate, which raise the pH and promote degradation of tacrolimus, (3) administration through jejunal tubes without adequate flushing with water, and (4) adsorption to the materials used in intravenous administration. In younger children and infants receiving low absolute amounts of tacrolimus administered intravenously through polyvinyl chloride tubing, adsorption may account for a large proportion of the dose and, therefore, wider fluctuations in the blood levels of this agent may be seen [9]. Apart from potential analytical problems, interday variation in tacrolimus levels in children may also be affected by the site and type of blood sample obtained. In our experience, venous samples are more reliable than samples obtained by lanset stick, which may be contaminated by higher tissue concentrations of tacrolimus or by erythrocyte lysate.

Therapeutic monitoring

The large interindividual variation in the pharmacokinetic profile of tacrolimus makes it difficult to define the optimal dosage schedule and underscores the importance of drug monitoring. Until recently, an enzyme-linked immunosorbent assay employing a mouse monoclonal antibody against tacrolimus was used to measure plasma concentrations [10, 11]. The major disadvantage of this method is that it does not accurately reflect drug levels because over 75% of the drug is found in red blood cells. Thus, as hematocrit frequently increases after renal transplantation, the plasma tacrolimus levels fall or the whole blood/plasma ratio increases at any given dosage. Conversely, the blood/plasma ratio may be reduced by anemia or by saturation of erythrocytes at high concentrations of tacrolimus. Moreover, the precision of the assay is highly dependent on blood sample preparation. Samples must be processed at 37° C because lower temperatures favor the partition of tacrolimus from plasma into red blood cells, thereby giving falsely lower plasma levels. A whole blood assay (Abbott IMx Tacrolimus Assay Kit, Abbott Laboratories, Abbott Park, IL 60064), which overcomes many of these abstacles, is currently undergoing clinical trials [12, 13]. This assay is rapid and has a sensitivity limit of 2.6 ng/ml and a coefficient of variation between 20% and 30%. Unpublished studies from our center show an intraassay and interassay variation of 9.1% and 9.6%, respectively. In general, levels ranging from 5 to 10 ng/ml are less reliable than those ranging from 10 to 45 ng/ml. Levels in the 1–10 ng/ml range may eventually represent the desired levels with

Table 4. Qualitative comparison of benefits and side effects of tacrolimus and cyclosporine in children with renal transplants

I.	Major advantages of tacrolimus
	Steroid sparing
	Excellent growth potential
	Less hypertension
	Rescue of cyclosporine-resistant rejections
II.	Minor advantages of tacrolimus
	Better graft survival
	Less hirsutism
	Less gingival hypertrophy
	Less neurological dysfunction
	Less metabolic acidosis
	Less hyperlipidemia
III.	Major disadvantages of tacrolimus
	Inreased viral infections
	CMV
	EBV
	Increased lymphoproliferative disease
IV.	Minor disadvantages of tacrolimus
	Increased acute rejections?
	More diabetogenic?
	Hyperkalemia?
	Hypomagnesemia?
V.	Similarities of tacrolimus and cyclosporine
	Nephrotoxicity
	Renal function

CMV, Cytomegalovirus; EBV, Epstein-Barr virus

Table 5. General features and major complications in 43 pediatric renal transplant recipients managed with tacrolimus for a mean period of 25 months

Early non-function	3/43 (7%)
Acute rejection	25 (58%)
LRD vs. CAD	14/21 vs. 11/22
Steroid-responsive	22 (88%)
ALG-responsive	2/3 (67%)
Allograft survival	
1 year	96%
3 years	85%
Allograft loss ($n = 4$)	Recurrent HUS at 10 days Rejection at 13.5 months MPGN type II at 29 months Non-compliance at 20.7 months
Renal function ($n = 39$)	
Serum creatinine	1.2 ± 0.6 mg/dl
Creatinine clearance (Schwartz)	75 ± 23 ml/min per 1.73 m^2
BUN	26 ± 11 mg/dl
Viral infections	CMV 6/43 (14%) EBV 4/43 (9%) EBV + CMV 2/43 (5%)
PTLD	5/43 (14%) 4 EBV 1 CMV + EBV
Transient diabetes mellitus	3/43 (7%)
Fasting serum cholesterol	178 ± 53 mg/dl
Hypertension	15/39 (38%)
1 drug	11 (mainly diuretic)
2 drugs	2
3 drugs	2

LRD, Living-related donor; CAD, cadaveric; PTLD, posttransplant lymphoproliferative disease; BUN, blood urea nitrogen; HUS, hemolytic uremic syndrome; MPGN, membranoproliferative glomerulonephritis

maintenance therapy, but an assay with improved sensitivity and precision characteristics is not yet available. Improved analytical methods would aid the performance of pharmacokinetic studies and permit investigations which correlate blood levels of tacrolimus and quantitative measures of immunological expression, rather than characterization by the presence or absence of rejection. Such studies would better define the blood level ranges which optimize immunosuppression while limiting toxicity.

It should be noted that tacrolimus may adsorb to polyvinyl chloride and polyurethane central venous and triple lumen catheters, as well as peripheral tubing and bags [9, 14]. Adsorption may not only result in significant loss in the amount of drug administered, but also in falsely elevated blood levels when blood is sampled from the same lines used for its infusion [15]. Drug adsorption may be reduced by using glass bottles and 5% dextrose to dissolve tacrolimus, and polyolefin syringes and administration sets.

Efficacy and benefits

The pharmacology and therapeutic efficacy and safety of tacrolimus in adults undergoing transplantation was recently reviewed [16, 17]; however, such information in children is limited. Nonetheless, aggregate data from a few uncontrolled pediatric studies attest to the immunological benefit of tacrolimus. This benefit is evidenced by improved survival with hepatic, small bowel, and multivisceral transplants [18], less severe rejection after cardiac transplantation [19], and rescue or conversion of hepatic, cardiac, and renal transplant recipients after inability of CsA to prevent rejection [20, 21].

The major and minor benefits of the tacrolimus regimen in children with renal transplants are listed in Table 4. Although graft survival is a major goal of immunosuppression, it should be noted that compared with a CsA/prednisone-based regimen, the 1-year allograft survival among 43 consecutive unselected children, including 51% cadaveric donors and 35% retransplants, was only marginally better at 96% (Table 5) [6, 21]. Results with the tacrolimus-based regimen, however, are remarkable because they include a higher proportion of children referred because of multiple failed transplants, high levels of panel-reactive antibodies, and complex urological disorders which characterized them as "high risk" for retransplantation. Renal function with the tacrolimus regimen is comparable to that obtained with CsA-based regimens. In addition, tacrolimus rescue of renal allografts was successful in 14 of 19 (74%) children managed at our center for CsA-resistant rejection; steroids were eventually stopped in 3 of these 14 children [21]. This group did not experience a greater number of infections or other complications. This potent immunosuppressive action of tacrolimus has enabled corticosteroids to be withdrawn in 93% of hepatic [18],

Table 6. Growth patterns (mean Z-score ± SD) in renal transplant recipients managed with tacrolimus[a]

Time	On steroids		Off steroids	
	≤12 Years (n = 11)	>12 Years (n = 4)	≤12 Years (n = 15)	>12 Years (n = 9)
At transplant	−1.9 (±12)	−3.0 (±1.1)	−2.7 (±1.3)	−2.3 (±2.6)
6 Months	−1.2 (±1.2)	−2.7 (±1.1)	−0.8 (±0.8)	−2.1 (±2.5)
1 Year	−0.4 (±1.1)	−2.1 (±1.1)	−0.02 (±1.7)	−1.6 (±2.4)
Follow-up 12±14 months (mean ± SD)	−0.42 (±1.7)	−2.0 (±1.1)	+0.92 (±1.6)	−1.4 (±2.3)
Mean change in height Z-score	+1.48	+1.0	+3.62*	+0.9

* $P < 0.01$ compared with other groups

[a] Z-score [23] = $\frac{\text{observed height} - \text{expected mean height for age [24]}}{\text{standard deviation of the height for age [24]}}$

83% of cardiac [19], and 62% of non-rescue renal recipients, without an increased risk for rejection or decrease in graft function. The number of children with renal transplants who can be withdrawn from steroids would be considerably higher if children with high panel-reactive antibody levels or with prior allograft loss due to recurrent glomerulonephritis were excluded from analysis. Only 3 additional children who developed mild rejection (2 due to non-compliance) returned to using steroids after initial withdrawal. The remaining 38% of our renal recipients were receiving an average prednisone dosage of 0.2±0.1 mg/kg per day at a mean follow-up of 25 months. This dosage is similar to the median prednisone dosage of 0.19 mg/kg per day at 24 months in children registered in the North American Pediatric Renal Transplant Cooperative Study [22].

Somatic growth in children with renal transplants has markedly improved after steroid withdrawal [6]. Further data (Table 6) indicate that the height standard deviation score, or Z-score [23], at a mean follow-up of 25 months is significantly greater in children ≤12 years of age managed by tacrolimus monotherapy. Although CsA monotherapy is also possible, concerns over allograft rejection and loss of renal function have resulted in the practice of using CsA/prednisone/azathioprine or CsA/prednisone immunosuppression in nearly all pediatric renal recipients in North America [22]. Another major advantage of the tacrolimus regimen is a marked reduction in the prevalence and severity of hypertension when the agent is used in conjunction with steroids or as monotherapy [6, 19].

Hirsutism and gingival hypertrophy are uncommon in children with renal transplants managed with tacrolimus [6, 19]. Although this is listed as a minor advantage, its importance, especially to females, cannot be minimized. Other minor advantages, including a lower incidence of metabolic acidosis or less hyperlipidemia, are not well established. Despite its lipophilicity, neurological symptoms attributable to tacrolimus are uncommon and are more likely to occur with intravenous use [18, 19]. Symptoms are usually mild, consisting of transient tremor, headache, insomnia, and paresthesia, which resolve with dosage reduction.

Toxicity

An improved toxicity profile over CsA-based immunosuppression would favor the use of tacrolimus, particularly in primary transplantation in adults in whom renal allograft survival rates are similar with either regimen [16, 17]. Such toxicity comparisons, however, may be invalid because the optimal dosage and blood levels of tacrolimus have not been established (partially due to difficulties with analytical assays and lack of a high correlation between dosage and blood levels), and because data comparing the two agents are not available from blinded randomized trials. Nevertheless, the therapeutic range of tacrolimus is relatively narrow, as adverse events are closely linked to increased blood concentrations and frequently resolve with dosage adjustment. Hence, regular blood level monitoring and monitoring of factors which affect blood levels is essential to avoid complications.

Complication rates obtained principally in adult liver recipients [8, 16, 17] may overestimate such events in adult recipients of renal grafts because of the more uniform bioavailability of tacrolimus in the latter group [25, 26]. Compared with CsA, tacrolimus has no unique side effects (Table 4) [6]. The major complications we have encountered with tacrolimus have been Epstein-Barr virus (EBV)-associated posttransplant lymphoproliferative disease (PTLD) and CMV infection.

Infections and PTLD

The most troubling complication, encountered mainly during the early period of our tacrolimus trial, was PTLD [6]. Although an increased susceptibility to PTLD was not noted among children undergoing cardiac transplantation [19], or in a large series of adults with renal allografts [27], this disorder occurred in 5 of 43 children (14%) with renal allografts at our institution [6, 21]. An even higher rate of EBV-related PTLD has been reported in children receiving tacrolimus for liver transplantation [18] or rescue [20].

Early symptoms or signs of PTLD are often mild and non-specific, and may include unexplained prolonged fever, lymphadenopathy, malaise, anorexia, abdominal

discomfort with or without vomiting or diarrhea, occult blood in the stool, leukopenia, thrombocytopenia, or atypical lymphocytosis. Seroconversion was determined by detection of antiviral capsid antigen (IgG or IgM), antiearly antigen and EB nuclear antigen, in conjunction with systemic illness and biopsy confirmed PTLD. These data, as well as immunocytochemical studies, established the polyclonal aspects of the tumor tissue and the role of primary EBV infection.

Analysis of the risk factors for PTLD in our 5 individuals revealed that a primary EBV infection was the most crucial predictor. All 5 cases of PTLD occurred among 9 children with EBV seroconversion between 5 and 36 weeks following transplantation. The donor (3 living-related) had a positive EBV titer in each case of seroconversion, including the 4 children who did not develop PTLD. Because EBV infection was evaluated only in symptomatic children rather than prospectively in all of our patients, we cannot reliably determine the incidence of EBV-induced PTLD. However, assuming a prevalence of 90% of EBV seropositivitiy in adults who comprised virtually all the donor pool in our patients, and an overall prevalence of 30% in children, we estimate that of 60% of children at risk of seroconversion after renal transplantation and tacrolimus immunosuppression, 10%–15% may develop PTLD. Preadolescent children may be more susceptible to PTLD because of the lower prevalence of EBV seroconversion prior to transplantation. Elevated tacrolimus levels in the blood (top quartile in 3 of 5) over several weeks prior to developing PTLD may comprise another risk factor for this disorder. It is noteworthy that EBV reactivation but no PTLD occurred in 1 of 3 children with positive EBV titers prior to transplantation. Occurrence of rejection (1 child with PTLD) and lack of acyclovir prophylaxis (2 of the 5 cases of PTLD or 2 of 9 cases of EBV seroconversion) did not appear to be important predisposing factors for PTLD. These clinical data strongly suggest that reactivation of latent infection in donor B-lymphocytes from the donor organ occurred secondary to tacrolimus-induced impairment of HLA-restricted $CD8^+$ cytotoxic T-lymphocyte responses which are essential in controlling latent EBV infection [28]. This would permit viral replication and EBV-induced B-cell activation as well as proliferation of other cell types. We speculate that delays in diagnosis and treatment of donor organ EBV reactivation lead to a combination of high viral titers, which may overcome any acyclovir suppressive effects, and together with a viral-induced inhibition of apoptosis may lead to graded levels of malignant transformation, as recently described [29]. A primary role of EBV in PTLD has recently been demonstrated [30]. The optimal dosages and efficacy of acyclovir or ganciclovir in prevention of EBV reactivation or primary infection have not been determined. Clearly, cases of PTLD have occurred in the setting of such preventive treatment after renal transplantation [31]. Apart from a lack of prior infection with EBV, children may be more susceptible to PTLD than adults because of higher dosages of steroids and tacrolimus per body weight and a more rapid turnover of gastrointestinal and other epithelial tissues. Although not supported by our data, utilization of OKT3 or treatment for recurrent rejection may also predispose or accelerate the time to presentation for PTLD [31–33].

Our latest strategy for reducing the incidence of PTLD consists of more rapid tapering of corticosteroids after the 1st posttransplant month when the incidence of rejection is very low. The role of lower blood tacrolimus levels than currently utilized in reducing the incidence of EBV-induced PTLD remains to be determined. The preventative value of CMV immune globulin, which is also enriched in EBV antibody, or the use of ganciclovir during the first 1–2 postoperative months in seronegative recipients of EBV-seropositive donor kidneys is currently being explored. Treatment of PTLD includes stopping tacrolimus, markedly reducing the prednisone dosage, and administering intravenous ganciclovir twice daily for 30 days at a dosage of 10 mg/kg per day, with appropriate adjustments for renal dysfunction. This is followed by oral acyclovir at a dosage of 1,500 mg/m^2 per day in three divided dosages until antiviral titers return to low levels for 2 months. Tacrolimus is slowly reintroduced and prednisone may be increased upon clinical and radiological resolution of the PTLD. A repeat but modified course of intravenous ganciclovir (one-half of the regular dose) may be given if antirejection therapy is required after the initial 30-day course of ganciclovir.

Of our 43 renal recipients managed with tacrolimus, 6 (14%) developed CMV infection and 1 patient developed disseminated varicella. This, however, does not represent an increased incidence of these infections compared with CsA immunosuppression in children [34] or tacrolimus use in adults with renal transplants [27]. The clinical presentation of CMV infection or reactivation was similar to EBV, but with more frequent gastrointestinal symptoms and elevation in serum liver enzyme levels. All 6 CMV infections occurred in CMV-negative recipients of CMV-positive donors and all 6 patients had received hyperimmune CMV immunoglobulin and oral acyclovir prophylaxis during the first 5 posttransplant months, when susceptibility to CMV is highest.

Treatment of CMV and varicella infection is identical to that of EBV or EBV-related PTLD. Although all antiviral courses were begun in the hospital setting, once symptoms and signs improved the antibiotic course was completed at home with minimal supervision by a visiting nurse. Treatment was well tolerated and all the children recovered fully. None of the renal recipients died or experienced graft dysfunction as a result of PTLD or other viral infections.

Other adverse events

Acute rejection episodes appear to occur more frequently with tacrolimus than a CsA-based regimen [6]. Rejections, however, are typically mild and easily overcome using the protocol shown in Table 2. The number of rejections is likely to decline with refinements in the immunosuppression protocol. New-onset diabetes mellitus occurs more frequently in the 1st posttransplant month of tacrolimus use than with CsA-based regimens [8, 35–37]. In adults, this disorder may be independent of concomitant use of steroids [37] or steroid dosage [38], and at 1-year post transplantation the incidence has been shown to be similar with ei-

ther immunosuppressive regimen [38]. A review of the current information suggests that tacrolimus may cause glucose intolerance by decreasing insulin secretion or through insulin resistance, or by a direct toxic effect on pancreatic β-cells [39]. Posttransplant diabetes mellitus occurred during the 1st month in 3 of 43 (7%) of our renal recipients in conjunction with high-dose prednisone (Table 5). However, the condition occurred in 3 of 12 subsequent transplants, bringing the frequency to 11%. All 6 children required insulin therapy. The disorder resolved in 2–5 months with steroid-tapering or withdrawal. Only 1 child required insulin therapy for a year; during this time the steroid dosages fluctuated markedly due to medical non-compliance-related rejection at 7 months post transplantation. In another study, however, 3 of 108 (2.8%) children with a variety of transplants or after tacrolimus rescue developed permanent diabetes mellitus [35].

A unexplained anemia (hematocrit <28%), which was responsive to exogenous erythropoietin, occurred in 25% of children with cardiac transplantation [19], but such anemia was not noted in children with renal transplants.

Nephrotoxicity

As with CsA, a main drawback of tacrolimus is nephrotoxicity. Reduced renal blood flow in humans [40] and animals [41, 42] may be mediated by endothelin-1 secretion by mesangial cells [42]. Direct tubular epithelial cell toxicity may also occur at high tissue concentrations of tacrolimus, presumably due to saturation of the tacrolimus-binding protein, with increased binding of the free agent to membranes and disruption of membrane integrity [43].

Nephrotoxicity has been reported in 50% of adults after hepatic transplantation [8] and in 52% of children with liver transplants during the early conversion period from CsA to tacrolimus [20]. In this setting, nephrotoxicity may result from hepatic dysfunction resulting in reduced metabolism and prolonged half-life of the tacrolimus which further increases the interpatient pharmacokinetic variability [44]. Children with cardiac transplants usually exhibit mild renal toxicity, which is manifested by an increase in mean serum creatinine concentration from 0.7 mg/dl pretransplantation to 0.9 mg/dl 3 months posttransplantation [19].

In adults with renal grafts, the relatively uniform bioavailability of tacrolimus, which approximates 22% of the oral dose, may diminish the risk of toxic levels and nephrotoxicity [43, 44]. In children, however, acute nephrotoxicity has mainly occurred in the setting of supratherapeutic blood levels of tacrolimus or with desirable blood levels during rescue of children with renal allografts managed with CsA. Despite discontinuing CsA during the rescue period, CsA continues to be mobilized from its large tissue distribution space, causing levels in the blood to fall slowly; simultaneously, tacrolimus levels may rise rapidly due to competitive inhibition of hepatic metabolism by both agents. The concomitant use of other agents that reduce the hepatic metabolism of tacrolimus may potentiate its nephrotoxicity (Table 3). Whereas renal function generally improves within 2–6 weeks of conversion of renal transplant recipients from CsA to tacrolimus, renal dysfunction may persist in liver recipients for much longer periods [20]. Long-term renal injury due to tacrolimus use has not been investigated.

Other renal-related complications include hyperkalemia, hypomagnesemia, and non-anion gap metabolic acidosis. As with CsA-induced metabolic acidosis, hyporeninemic hypoaldosteronism and renal dysfunction may also play a role in the pathogenesis of this disorder with tacrolimus use [45]. Hyperkalemia does not appear to be related to the level of renal dysfunction or to the severity of metabolic acidosis. This disorder is more common in the first few months of therapy and responds well to a brief course of fludrocortisone acetate (Florinef) at a starting oral dose of 0.1 mg once or twice daily. Mild renal magnesium wasting has also been noted and on occasion may require brief supplementation with magnesium gluconate or magnesium sulfate.

Summary

Tacrolimus is a highly effective immunosuppressive agent in children with renal allografts, and may also control a high proportion of CsA-resistant acute rejections in this population. Other benefits include absence of hirsutism and gingival hypertrophy, minimal hypertension, and steroid withdrawal, which promotes somatic growth. With the possible exception of an increased incidence of EBV-related PTLD, which follows a benign course, toxicity appears to be comparable to CsA-based regimens. Because of the high potency and interindividual pharmacokinetic variability of tacrolimus, attention to factors that influence its bioavailability and close therapeutic monitoring are essential to guide dosage adjustments and optimize efficacy and safety.

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