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Chapter 7

Overview of FK 506 in transplantation

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Introduction

In 1987, Ochiai *et al.*, reported the immunosuppressive qualities of a new immunosuppressive agent, FK 506, isolated from the fermentation broth of a soil fungus, *Streptomyces tsukubaensis*. Extensive *in vitro* studies, demonstrated the effectiveness of FK 506 in suppressing mixed lymphocyte cultures, apparently by inhibiting IL-2 synthesis following alloactivation (Kino *et al.*, 1987). The receptor for FK 506 has been identified, and has been characterized as a peptidyl-prolyl *cis-trans* isomerase (Harding *et al.*, 1989).

The background for the clinical development of FK 506 was based on a number of animal models which have shown a marked ability of the drug to prevent rejection following various types of organ transplants (Starzl *et al.*, 1987; Todo *et al.*, 1988; Murase *et al.*, 1990a,b), as well as to prevent the development of graft-versus-host disease (GVHD) following bone marrow transplantation (Markus *et al.*, 1991a). More interestingly, FK 506 possesses the ability to reverse ongoing rejection in animal models, as well as established GVHD after bone marrow transplantation (Markus *et al.*, 1991b). These properties continue to be evident during clinical testing of FK 506 (Starzl *et al.*, 1989).

This chapter will attempt to summarize the results of all human transplantation models in which FK 506 has been utilized as either

'rescue' therapy and/or 'primary' therapy, including liver (Starzl *et al.*, 1989; Fung *et al.*, 1990; Todo *et al.*, 1990), kidney (Starzl *et al.*, 1990; Todo *et al.*, 1990; Shapiro *et al.*, 1991), heart (Armitage *et al.*, 1991) and bone marrow transplantation (Fung *et al.*, 1990), at the University of Pittsburgh.

Methods

Study design

The trials in liver, kidney, heart and bone marrow transplantation were conducted at the University of Pittsburgh, Presbyterian University Hospital, Children's Hospital of Pittsburgh and the Veterans Administration Medical Center, with the approval of the respective Institutional Review Boards. Informed consent was obtained from patients or their appointed guardians.

Patient profiles

In the liver study, patients were treated with FK 506 as part of three studies, one being the rescue study, in which 57 patients were entered for the diagnosis of acute rejection, while 116 patients were converted from cyclosporin A (CsA) to FK 506 for chronic rejection. In the primary liver transplant group, 110 patients were treated with FK 506 and low-dose steroids, as the baseline immunosuppression following liver transplantation. A subsequent study involved a total of 81 patients, prospectively randomized to either FK 506 or CsA as the baseline immunosuppression following liver transplantation.

In the kidney study, patients were treated with FK 506 as part of two studies, one being the rescue study, in which 21 patients were entered for the diagnosis of rejection. In the primary kidney transplant group, 202 patients were treated with FK 506 and low-dose steroids, as the baseline immunosuppression following kidney transplantation. A randomized trial comparing FK 506 and CsA primary kidney transplantation included 26 patients in both arms.

In the heart study, patients were also divided into two groups. In the first group, 30 patients were treated with FK 506 for primary immunosuppression, while in the second group, ten patients were converted to FK 506 because of persistent rejection.

In the bone marrow study, patients were entered as part of a rescue study, in which 14 patients were treated with FK 506 for evidence of persistent manifestations of graft-versus-host disease, unresponsive to conventional treatment protocols.

Diagnostic evaluations

For patients who were experiencing organ dysfunction, the final categorization of dysfunction was based upon clinical, biochemical and/or histopathological findings. For all patients, either as primary or as rescue therapy, cause(s) or organ dysfunction were carefully sought, the workup being customized to the organ or tissue transplanted. Ultrasonic determination of vessel patency and radiographical evaluation of the biliary or urinary system were used to rule out a technical or mechanical defect. Angiography was performed when indicated. Appropriate viral cultures and stains were used to detect viral infections.

Protocol biopsies were utilized in the evaluation of efficacy of FK 506 therapy. All biopsies were blinded and interpreted by a single experienced transplant pathologist (A.J.D.). Biopsy specimens were fixed in neutral buffered formalin and stained routinely with haematoxylin and eosin, trichrome and reticulin stains. The criteria used for pathological diagnosis have been defined in previous reports (Billingham *et al.*, 1979; Demetris *et al.*, 1990).

Timing and details of therapy

Initiation of treatment with FK 506 was done in the hospital and was given initially as a parenteral dose, followed by conversion to an oral dose. The initial parenteral dose of FK 506 was 0.075–0.15 mg/kg, given intravenously. This was continued until the patient was able to ingest the oral form of FK 506. Generally, oral dosages of FK 506 were given at 0.30 mg/kg/day, in two divided doses. Dose adjustments of FK 506 were based upon monitoring of serum trough levels by enzyme linked immunosorbant assay (ELISA) (Tamura *et al.*, 1987) to achieve a 12-h trough level between 1.0–2.0 ng/ml, and also by adjustment according to clinical or biochemical parameters.

Evaluation of response

Periodic determinations of liver and kidney functions, including total bilirubin (TBIL), serum glutamic transaminases, SGOT and SGPT, alkaline phosphatase, blood urea nitrogen (BUN) and serum creatinine were performed. All values are expressed as the value \pm 1 s.d. Protocol biopsies were obtained after initiation of FK 506 therapy.

Liver transplantation

Rescue therapy

In this population of 173 patients, of whom many were critically ill at the time of FK 506 conversion, there were 14 deaths (8.1%). The causes of

death were numerous, but the incidence of mortality was directly correlated with the medical condition of the patient at the time of FK 506 conversion. Sepsis was the cause of death in four patients. Three patients died of haemorrhagic complications. Three patients died of metastatic carcinoma following transplantation. In two patients, retransplantation was not considered an option for the failing liver allograft. One patient was started on FK 506 with pathological findings of late chronic rejection, and died of technical causes during an attempted retransplantation. In one death no cause of death could be determined. This patient died at home and had been off FK 506 for 4 months when she died. She had renal failure and was on dialysis prior to and after discontinuation of FK 506 therapy.

The biochemical response of the liver allografts to FK 506 was analysed by classifying patients either into acute or chronic rejection, dependent upon the principal histopathological findings. For the 57 patients who were treated for acute rejection, documented on liver biopsy or as judged by biochemical and clinical parameters, the TBIL, SGOT, and SGPT values prior to FK 506 were: 4.68 ± 5.91 mg/dl, 240 ± 431 iu/l, and 292 ± 383 iu/l, respectively. These values fell, by the sixth month to: 0.76 ± 1.41 mg/dl, 98 ± 163 iu/l, and 90 ± 128 iu/l, respectively.

Patients with an entrance diagnosis of chronic rejection also had a beneficial response to FK 506. For the 116 patients treated for this specific indication, the total bilirubin fell to normal values (pre-FK 506, 5.07 ± 8.16 mg/dl; 6 months, 0.99 ± 1.47 mg/dl) while the average transaminase values were still slightly elevated above normal values (pre-FK 506, SGOT/SGPT, 200 ± 175 iu/l/ 275 ± 223 iu/l; 6 months, SGOT/SGPT, 44 ± 72 iu/l/ 101 ± 68 iu/l).

A clinicopathological study of the results of conversion of liver allografts from CsA to FK 506 immunosuppression revealed that the biochemical improvement seen above was correlated with histopathological improvement (Demetris *et al.*, 1991). With both acute and chronic rejection, the biochemical improvement occurred earlier and in greater proportion than the pathological findings. Those patients with acute rejection fared better than those with chronic rejection, with a higher response rate. In those patients with chronic rejection, the liver function studies and the degree of bile ductular injury was significantly worse among those who failed than among those patients who responded.

Primary therapy

Of the original 110 primary liver transplant patients in the series, a total of 99 (90%) were alive at 12 months. These results were statistically better than those of the 325 CsA-treated control group, which had a 1-year patient survival of only 77%. The corresponding graft survival was also statistically better, with 83% of the FK 506 grafts surviving at 1 year, compared with 68% for the CsA group. The rate of retransplantation was

only 6%, over one-half less than the 15% retransplant rate seen with the CsA control group. The 60-day mortality figure for FK 506-treated patients (6.7%) was statistically less than that of CsA (16.5%).

During the follow-up period, 50% of all recipients were taken off steroids and were maintained on single drug immunosuppression with FK 506. Yet 52.8% of all patients were rejection free during the entire period of study. The majority of rejection episodes were mild and easily controlled with a single dose of bolus steroids (either methylprednisolone or hydrocortisone). Only 17.8% of the rejection episodes required further steroid treatment in the form of a steroid taper or additional steroid boluses. In addition, only 11.2% of the patients required anti-human CD3 (OKT3).

The incidence of serious infections, in spite of the potency of FK 506, does not appear to be alarming. The incidence of serious infections was about 50% less than that seen with a historical group of patients given CsA. Of note, is that the incidence of cytomegalovirus infections did not appear to be increased, when compared to patients on CsA.

Prospective randomized study

Based upon the encouraging results of the preliminary FK 506 experience, a study was started, comparing the use of FK 506 and CsA, along with steroids, in a prospective, randomized fashion, in patients undergoing primary liver transplantation. Eighty-one liver transplant recipients were randomized to either FK 506 (41 patients) or CsA (40 patients), following completion of the liver transplant. A single bolus of 1 g methylprednisolone followed by a daily dose of 20 mg methylprednisolone was the baseline steroid therapy for both groups of patients. Biochemical and histopathological parameters were monitored in order to determine the effectiveness of either therapy in preventing rejection. Rejection episodes were treated with a single bolus of 1 g methylprednisolone. If this treatment failed to reverse the rejection episode, a total of 50 mg OKT3 was administered. Those CsA patients who failed to respond to therapy were converted to FK 506, in an attempt to rescue the dysfunctional graft. With the ability to rescue CsA randomized dysfunctional grafts with FK 506, the patient and graft survival were essentially the same.

The median follow-up for both the CsA and FK 506 groups was 345 days (range 256–446 days). The 6-month-patient survival rate was 95% for the FK 506-treated group, while the corresponding value for the CsA-treated group was 89%. The corresponding graft survival was 93% for the FK 506-treated group, while the corresponding value for CsA-treated group was 81%. The 12-month-graft survival rate was 90% for FK 506, and 70% for CsA. Two patients in the FK 506-treated group and seven patients in the CsA-treated groups were retransplanted. The mortality

associated with retransplantation was 50% in both groups. The total percentage of patients in the FK 506 group who were rejection-free during the entire length of follow-up was 53.7%, while that for CsA was 13.3%. The mean days to the first rejection was 21.5 days for the FK 506-treated group, and 9.9 days for the CsA-treated group. OKT3 for treatment of the original allograft was used in 30% of CsA-treated patients, compared with 20% of FK 506-treated patients. Steroid boluses averaged 0.99 times in the CsA-treated group, while this figure was 0.50 times in the FK 506-treated group. 72.6% of the CsA-treated patients were converted to FK 506, an average of 20 days after liver transplantation. The reasons for conversion were: persistent ischemic injury (5), patient dropout (1), Rh incompatibility with haemolysis (1), and steroid resistant or OKT3-resistant rejections (21).

Renal function in both groups of patients was assessed by the requirement for haemodialysis and the serum creatinine at monthly determinations. Haemodialysis was initiated in six CsA patients while still on CsA, while three other CsA patients required haemodialysis during the period of conversion to FK 506. In the FK 506-randomized group, four patients were placed on haemodialysis during the post-transplant period. The comparative incidence for haemodialysis requirement between the FK 506 and CsA groups was 10% and 21.6%, respectively. Long-term haemodialysis (after 3 months post-transplant) was required by one patient in each group.

The incidence of opportunistic infections was essentially the same for both groups. Patients who were randomized to CsA had a 22.5% incidence of cytomegalovirus (CMV) infections. This compared to a 22.0% incidence for patients on FK 506. In the 13 CsA patients who were not switched to FK 506, the incidence of CMV was 23% (3/13), and only one of the three patients received OKT3. In FK 506 patients, three of a total of nine cases of CMV occurred in patients who had previously received OKT3.

The severity of hypertension was assessed by the need for antihypertensive medications following transplantation. The incidence of hypertension in the overall CsA-randomized group was 52.9% versus 26.9% for the FK 506-treated group ($P < 0.01$), at 3 months post-transplant. This figure did not change appreciably over the follow-up period; at the 12-month follow-up period, the corresponding hypertensive incidence was 48% for CsA and 33.3% for FK 506. The incidence of hypertension in the 14 patients, who were on CsA at the 3 month post-transplant period was 64.2%. The 12-month figure was 72.7% for those patients still on CsA, while the conversion group had an incidence similar to those given FK 506 from the start (36.4%).

The need for insulin therapy was evaluated by determining those patients who required insulin at the 3-month period following transplantation. There were no statistically significant differences between the two

groups of patients. 17% of the patients in the FK 506 group required insulin at 3 months post-transplant. For the CsA group, 17.5% of the patients required insulin at the same time point.

Both FK 506 and CsA administration have been associated with side-effects, many of which are similar. The percentages and severity of patients experiencing treatment-related adverse reactions were recorded. This included evidence of neurotoxicity: trembling, paresthesias, insomnia, irritability, hyperkinetic behaviour, dysarthria, seizures, and coma. The incidence of side-effects was essentially the same in both groups.

The results of the current randomized study compare favourably with previously reported results using FK 506, and therefore do not appear to represent a bias in the performance of the study. It is important that the results of the current ongoing randomized trial are comparable to those results obtained in the historical series. The current results of patient and graft survival are as good, if not better, than those figures obtained in the past. One would expect that both graft and patient survival would be better in the randomized trial since high-risk patients are removed from randomization. The randomized FK 506 liver patients were compared to the survival curves of 271 non-randomized FK 506 recipients and 813 CsA recipients during the period of time corresponding to the utilization of Viaspan (Dupont). The 1-year patient survival for the high-risk FK 506 liver recipients not entered in the randomized trial approaches 82%, while graft survival is 76%. This compares to our historic CsA patient and graft survival of 77% and 68%, respectively. The improvement of the current randomized CsA group over the historic group may, in part, be, related to the ability to convert patients on CsA to FK 506.

Kidney transplantation

Rescue therapy

A total of 21 patients were converted from CsA-based immunosuppression to FK 506-based immunosuppression for persistent kidney rejection. One death was encountered. Of the 21 patients, ten were classified into late rejection episodes (> 60 days), while 11 were treated early in the post-transplant course (< 60 days). Seven of the 11 early rescues were successful, in contrast to only four of ten late rescues. Most of the failures of FK 506-rescue therapy in this group of patients were in patients who had chronic glomerulosclerosis and chronic rejection on biopsy, prior to FK 506 rescue. In those patients with acute cellular rejection, the results were better. The serum creatinine at the time of conversion, was also correlated with the success of therapy. Four of five (80%) patients with a serum creatinine < 3.0 mg/dl have good renal function, while only seven of 16

(44%) patients with a pre-conversion serum creatinine > 3.0 mg/dl have a functioning kidney. The overall serum creatinine prior to FK 506 conversion in the 11 successful switches was 3.70 ± 2.15 mg/dl, excluding the serum creatinine values of four patients who were on dialysis at the time of FK 506 conversion. The average creatinine after FK 506 switch was 2.84 ± 1.40 mg/dl, with all 11 grafts functioning.

Primary therapy

On March 27, 1989, a trial utilizing FK 506 and low-dose steroids was introduced at the University of Pittsburgh. A total of 411 kidney transplants have been performed during the period between 3/27/89 and 1/31/91. Twenty-nine kidneys were transplanted into patients who were recipients of other organ transplants. The results of these patients were limited by other factors such as patient survival and graft function of other organs, primarily that of the liver, and were excluded from further analysis. A study of the remaining 382 kidney transplant patients included 202 patients given FK 506 and low-dose steroids and 180 patients given CsA, low-dose steroids and azathioprine.

The average age of the recipients was 39 years; 11.3% of recipients were over 60-years old and 8.4% were in the paediatric age range. Approximately two-thirds of the patients in both groups were receiving their first kidney transplant. Fifteen per cent of patients were considered high antibody status (panel reactive antibodies (PRA) $> 40\%$), placing them in a high-risk category for kidney transplantation. A slightly higher percentage of patients with living related transplants were given CsA (14% CsA versus 5% FK 506), while paediatric donors accounted for a higher percentage of the organs used in the FK 506 group (22% FK 506 versus 15% CsA). The mean follow-up was 11.3 ± 7.5 months. The overall patient survival at 1 year was 94.3% for the CsA group and 90.6% for the FK 506 group. The causes of death were similar between both groups, with cardiac causes accounting for 30% of deaths, sepsis for 26% of deaths and gastrointestinal complications for 22% of deaths.

For those patients receiving their first kidney transplant, the patient survival was essentially the same (92.8% CsA versus 92.7% FK 506). The graft survival at 1 year for these patients was 81.5% for the CsA group and 75.3% for FK 506. For the low immunological risk patients (PRA $< 40\%$), the patient survival was 94.1% for CsA and 91.1% for FK 506, while the corresponding graft survival for the CsA was 81% versus 76% for FK 506.

Prospective randomized study

A randomized trial of FK 506 with low-dose steroids versus CsA with low-dose steroids was initiated in April, 1990. At this time, patients who were

considered as low-risk candidates for kidney transplantation, i.e. receiving their first kidney transplant without significant known medical problems, and with low PRA, were randomized to either FK 506 or CsA with low-dose steroids. Patients who did not fulfill these criteria were considered high-risk patients and received FK 506 and low-dose steroids as part of a non-randomized protocol. When the results of patient and graft survival were examined, breaking down the randomized from the non-randomized patients, the effect of the non-randomized group on the FK 506 statistics could be seen. The patient and graft survival of the randomized patients were essentially the same (see Figs 7.1 and 7.2). The 1 year patient survival in the randomized CsA group was 92% and 96% for the FK 506 group. The corresponding graft survival was 79% and 81%. By shifting the high-risk patients into the FK 506 non-randomized trial, the overall patient and graft survival were lower in the FK 506 group. The 1-year patient and graft survival for the FK 506 non-randomized group was 90% while that for the CsA was 95%. More importantly, because of higher numbers of retransplant cases in the FK 506 group (32% for FK 506 versus 21% for CsA), the

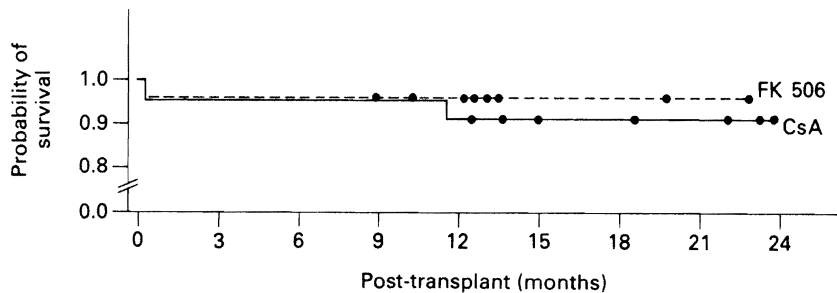


Fig. 7.1. Patient survival curves for FK 506 and CsA kidney transplant randomized patients are shown. Dots denote censored times.

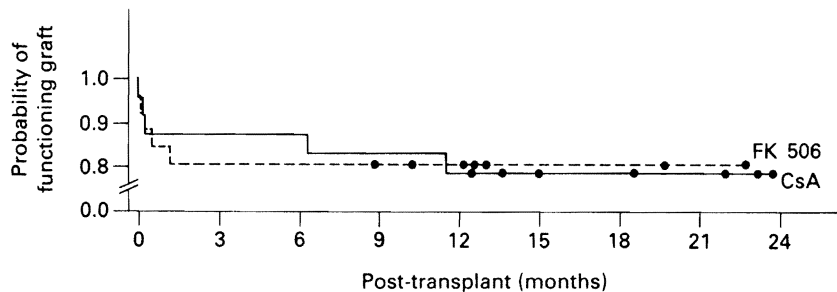


Fig. 7.2. The corresponding graft survival curves for the FK 506 and CsA kidney transplant randomized patients are shown. Dots denote censored times.

overall graft survival were somewhat less in the FK 506 group (74%) when compared to the CsA group (82%). The rates of rejection in the overall groups were similar, 57% in the FK 506 group and 54% in the CsA group.

The randomized trial was designed to evaluate not only patient and graft survival, but also to assess the effect of either FK 506 or CsA on other parameters such as the incidence of hypertension, infection, requirement for augmented immunosuppression, rates of rejection, and steroid requirements. Fifty-two patients were enrolled in the study, 26 randomized to both arms. The incidence of biopsy documented rejection was 73% in the CsA group and 46% in the FK 506 group ($P < 0.05$). Moreover, the requirement for OKT3 was 50% in the CsA group and 19% for the FK 506 group ($P < 0.03$). Thirty-five per cent of the CsA group required conversion to FK 506, while 4% of the FK 506 were considered treatment failures and required addition of azathioprine to control rejection. The rates of CMV infection were essentially the same, 15% for CsA and 12% for FK 506. The overall renal function was similar in both groups, with a mean serum creatinine of 1.9 mg/dl, and a mean blood urea nitrogen of 28 mg/dl for CsA and 26 mg/dl for FK 506. While over one-third of FK 506 patients were off steroids at the time of analysis, no CsA patient was steroid free ($P < 0.01$). While only 29% of patients on CsA were given no antihypertensive medications, over one-half (52%) of patients on FK 506 were free of antihypertensive medications ($P < 0.05$).

Heart transplantation

Rescue therapy

Ten patients were converted from CsA to FK 506 between 3 and 50 months post-transplant. The findings of persistent heart rejection defined by a $> 2+$ grading of the endomyocardial biopsy by the Billingham criteria (Billingham *et al.*, 1979), included mononuclear cell infiltration, arteritis and in some instances, interstitial fibrosis. All patients had failed conventional immunotherapy, including at least two courses of antilymphocyte preparations, and two courses of augmented steroids during the preceding 6 months. The grading of endomyocardial biopsies, prior to conversion of FK 506, was 2.70 ± 0.48 . Using the same criteria, the mean value of the follow-up biopsies after FK 506 was graded at 0.70 ± 0.67 ($P < 0.01$). The mean prednisone dose prior to FK 506 conversion was 14 mg/day, after FK 506 conversion this fell to 5.5 mg/day. One death occurred during the period of follow-up in a patient with disseminated aspergillosis.

Primary therapy

Thirty patients received FK 506 from the outset following heart transplantation. Eight patients were on circulatory assist devices prior to heart transplantation. Follow-up ranged from 1 to 10 months. Four patients have died, with an actual patient and graft survival of 87%. One patient with known pulmonary hypertension died on the third post-transplant day from right heart failure. One patient, with pre-existing lung disease and bronchiectasis, died from pulmonary infection, while two other patients died of sudden deaths, without a known cause. The rejection-free rate within the first 90 days was 60%. Only one patient required OKT3. Heart function was excellent in all patients. The average left ventricular rejection fraction, determined by gated nuclear scans or echocardiography, was 70% (range 58-75%).

Bone marrow transplantation

Rescue therapy

Fourteen patients with manifestations of chronic GVHD following bone marrow transplantation were placed on FK 506. All patients were on or had been on high doses of CsA and steroids. Seven patients had an original diagnosis of chronic myelogenous leukaemia, three were given bone marrow transplants for acute lymphoblastic leukaemias, one had aplastic anaemia, one for Burkitt's lymphoma and two had acute myelogenous leukaemias. All grafts were taken from human leukocyte antigen (HLA) identical siblings. The most common sites of involvement are skin and liver, followed by lung, gastrointestinal and musculoskeletal. The most objective parameters to evaluate response to FK 506 have been those with liver and skin involvement. The mean time after bone marrow transplantation to the time of FK 506 therapy was 17.7 months, and the mean follow-up was 5.8 months.

Of the 11 patients with liver involvement, five were referred for consideration for liver transplantation. Two of these eventually required liver transplantation, but both died. One died from sepsis following liver transplantation, while the other patient failed to awaken after transplantation, having been in stage IV coma prior to transplantation. Two other patients died, one of unknown causes at home, and the other from respiratory failure from pre-existing severe bronchiolitis obliterans. The other six patients had a marked response to FK 506-rescue therapy.

In the 11 patients with skin involvement, five have improved, while three with scleroderma like involvement have stable skin lesions. One of the patients dropped out of the study while two other patients died, one

from pulmonary aspergillosis and the other of unknown causes.

Of the six patients with moderate to severe obliterative bronchiolitis, two patients died from worsening lung disease. The remaining four patients have stable or slightly improved pulmonary function. The remaining organ system involvement of musculoskeletal and the gastrointestinal tract have not shown progression during FK 506 therapy.

Limitations

Adverse reactions requiring treatment or adjustment of FK doses can be categorized into four primary areas. These are (i) alterations in kidney functions; (ii) alterations in glucose metabolism; (iii) neurotoxicity; and (iv) susceptibility to infection or malignancy.

Alterations in kidney function are manifested by electrolyte abnormalities and changes in glomerular filtration, as evidenced by changes in serum creatinine. Hyperkalaemia is seen in 35% of patients, following administration of FK 506. Treatment of hyperkalaemia is generally with potassium-binding resins and potassium-restricted diets. Addition of a synthetic mineralocorticoid, Florinef, relieves the hyperkalaemia, by increasing potassium excretion by the kidney. Decrements in renal blood flow have been documented by nuclear medicine studies. The filtration fraction generally remains the same. Causes of altered renal function in transplant patients are multifactorial, and include: peri-operative hypotension, use of nephrotoxic antibiotics and a degree of pre-existing renal dysfunction. The alteration in renal function seen in patients on FK 506 is similar to that seen in patients on CsA. These changes are responsive to reduction in doses of FK 506. The incidence of renal failure requiring chronic haemodialysis is of the order of 4%, based on studies of liver transplant patients, although no patients have required maintenance haemodialysis following heart transplantation. The progression of chronic renal failure to dialysis-requiring renal failure is not known.

Alterations in glucose metabolism are the result of changes in peripheral sensitivity to insulin and/or changes in the response of the islet cells to hyperglycaemia. The incidence of new onset diabetes, i.e. those patients requiring insulin, is approximately 15% in FK 506 transplant patients. The incidence of new onset diabetes in other immunosuppressive regimens, incorporating CsA or azathioprine, is approximately 20%. The long-term consequence of insulin requirement in transplant patients, towards the development of diabetic complications is not known.

Rare but severe instances of neurotoxicity have been reported following FK 506 administration. Expressive aphasia has been seen in four liver transplant patients, although this has not been seen in any other types of FK 506-treated individuals. New onset seizures have also been reported in liver transplant patients, especially during the peri-operative transplant

period. The susceptibility of such patients to changes in serum electrolytes has been previously reported. New onset seizures have not been reported in other patients treated with FK 506.

Post-transplant lymphoproliferative disease (PTLD) is an abnormality of lymphocyte proliferation in a setting of an immunosuppressed patient. The spectrum of PTLD can range from a benign lymphoid proliferation such as a mononucleosis syndrome to a frankly malignant lymphoid tumour. PTLD has been associated with all types of immunosuppressive therapy. The incidence of PTLD in the cyclosporin era is generally estimated between 2 and 4%. The median time following transplantation to the development of PTLD is 6 months; the majority of these tumours occur within 12 months following transplantation.

A total of 16 patients have developed *de novo* PTLD lesions while on FK 506 therapy. Seven of these patients died, although PTLD was associated with death in only five cases. The remaining nine patients had relatively mild forms of PTLD, three of these had a mononucleosis syndrome, and presented with sore throat and tonsillar enlargement. Treatment with lowering immunosuppression and intravenous acyclovir proved to cure all of them. In the remaining six patients, three required operative procedures (two small bowel resection, one liver resection) which were directly related to PTLD, while the other three were treated by a reduction of immunosuppression only. The incidence of *de novo* PTLD following initiation of FK 506 therapy is 1.4%. All of the cases of PTLD occurred within the first year following initiation of FK 506, with the median time from FK 506 therapy to onset of disease being 4 months. FK 506 shows no evidence of any increase in the risk of developing or succumbing to PTLD, when compared to previously quoted figures on the incidence of PTLD, based on other immunosuppressive regimens. No patients treated with FK 506 for non-transplant indications have developed any malignancies.

Cytomegalovirus infections are considered the most common opportunistic infection in the transplant patient. Several factors determine the severity and development of CMV infections. The seronegativity and use of intensive immunosuppression are considered major contributing factors. The incidence of CMV infections in the FK 506-treated transplant patients is 20%. This figure is similar to that seen in transplanted patients on CsA. No patients treated with FK 506 for non-transplant indications have developed CMV infections.

Discussion

Cyclosporin-based immunosuppression significantly enhanced both patient and graft survival in all solid organ transplants, when compared to the era of azathioprine and steroids (Starzl *et al.*, 1990). Its use in bone marrow transplantation has decreased the incidence and severity of

GVHD (Sullivan *et al.*, 1988). Nevertheless, most centres experience an unacceptably high complication rate related to ongoing GVHD or rejection. These immunologically related complications occur in over 70% of all cyclosporin-treated patients. In addition, the sequela of over-immunosuppression in attempts to treat rejection or GVHD, such as the use of excessive steroids and antilymphocyte preparations, are fraught with a high incidence of infectious complications. It stands to reason that a baseline immunosuppressive agent which allows for less incidence of rejection or GVHD, and easier treatment, would decrease both graft and patient loss. From the results of our studies presented here, the use of FK 506 in transplantation has these advantages. FK 506 appears to not only decrease the absolute incidence of rejection episodes, but makes the treatment of rejection simpler.

The ability of a new immunosuppressive agent to be dose adjustable for treatment of acute rejection, chronic rejection or GVHD, would represent an important asset; this ability has only been ascribed to steroids in the past. FK 506 can be used in this manner. In fact, the first response to a developing rejection, is to increase the dose of baseline FK 506. In rescue therapy, the marked ability of FK 506 to reverse acute rejection in both kidney and heart rejection, and both acute and chronic rejection in liver transplantation, and with chronic GVHD in bone marrow transplantation has not been seen with any immunosuppressive agent in the past. While the mechanism by which FK 506 is able to do this, is not known, it would appear that it would entail mechanisms other than simply inhibition of IL-2 synthesis.

The limitations of FK 506 have been defined. In chronic rejection, the ability of an organ to reverse the stigmata of chronic inflammation appears to determine the effectiveness of FK 506 rescue therapy. With the development of obliterative arteriopathy, or the absence of epithelial structures, e.g. biliary structures or kidney tubules, the ability of FK 506 to reverse organ dysfunction is limited. On the other hand, acute cellular rejection appears to respond well to initiation or adjustment of FK 506 therapy.

Prospective, randomized trials comparing FK 506 therapy with cyclosporin-based immunosuppression are currently underway. The preliminary results of the study being performed at the University of Pittsburgh in liver transplantation, are encouraging. Multicentre trials are also underway, and preliminary reports are also encouraging. A well defined endpoint, other than patient or graft loss, should be utilized, since the data presented here also suggests that a conversion to FK 506, will allow for allografts in danger of being lost to rejection, to be salvaged.

Other randomized trials in kidney, heart and bone marrow transplantation are also underway. The results of the randomized kidney transplant trial at the University of Pittsburgh has demonstrated a benefit of FK 506 in several important secondary endpoints, e.g. incidence of hypertension,

although the primary endpoints of patient and graft survival are similar. One other centre, in Japan, has had some early experience with FK 506 for primary kidney transplantation. Between July and September 1990, 37 patients (32 living related and five cadaveric allografts) were enrolled in a study utilizing a fixed dose of 0.15 mg/kg/day intravenously followed by a fixed oral dose of 0.30 mg/kg/day. No attempts to adjust FK 506 doses to levels were made until an adverse event occurred. With follow-up of 5-8 months, all patients were alive, and all grafts functioning. Twenty-five per cent of patients were converted to other immunosuppressive drugs because of adverse events. Rejection was noted in 32% of the patients although no patients required OKT3. Nephrotoxicity was seen in 30% of patients, and responded in 92% of patients to a decrease in FK 506 dose. Adverse reactions to the drug were proportional to high levels of FK 506.

The toxicity profile of FK 506 is proportional to the efficacy of the drug. The major side-effects of the drug are similar to cyclosporin, with dose-related nephrotoxicity and neurotoxicity. Other known complications of immunosuppression, including diabetogenesis, infectious and malignant complications, are no greater with FK 506 than with other immunosuppressive therapies. As with any new agent, details regarding nuances of dose administration and adjustments, are continuing to be refined. In fact, it has been recently reported that FK 506 dosing must be adjusted to liver function and that attention to drug levels may be helpful in avoiding overdosing (Abu-Elmagd *et al.*, 1991; Starzl *et al.*, 1991).

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