1322

FK506 in clinical organ transplantation

Fung JJ, Abu-Elmagd K, Todo S, Shapiro R, Tzakis A, Jordan M, Armitage J, Jain A, Martin M, Bronster O, Stieber A, Kormos R, Selby R, Gordon R, Starzl TE. FK506 in clinical organ transplantation. Clin Transplantation 1991: (Spec. issue) 5: 517–522.

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Key words: transplantation – FK506

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Accepted for publication 2 August 1991

Introduction

The background for the clinical development of FK506 was well documented by Starzl in the opening remarks to a satellite symposium on FK506, held in Barcelona, Spain, in November 1989, of European Society of Organ Transplantation (1). Following the initial description of its discovery in 1987 by Ochiai and coworkers, extensive *in vitro* studies demonstrated the effectiveness in suppressing mixed lymphocyte cultures, presumably by inhibiting IL-2 synthesis following alloactivation (2, 3). *In vivo* studies using a number of animal models have shown a marked ability to prevent rejection following various types of organ transplants (4–7). More interestingly, FK506 possesses the ability to reverse ongoing rejection in animal models (7–8). These properties served as the initiative to pursue clinical testing of FK506.

The objective of this manuscript is to summarize the current experience of FK506 in human solid organ transplantation at the University of Pittsburgh. In addition to the efficacy of FK506, a delineation of the limitations, toxicities and benefits, is also provided.

Methods

Study design

The trials in liver, kidney and heart 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. No attempt to chronologically report the results of the various studies is made, nor is this report to be taken as a representation of the total experience at the University of Pittsburgh.

Patient profiles

In the liver study, patients were treated with FK506 as part of two studies, one being the rescue study in which 57 patients were entered for the diagnosis of acute rejection, while 116 patients were converted from cyclosporine to FK506 for chronic rejection. In the primary liver transplant group, 125 patients were treated with FK506 and low-dose

steroids as the baseline immunosuppression following liver transplantation.

In the kidney study, patients were treated with FK506 as part of two studies, one being the rescue study in which 27 patients were entered for the diagnosis of rejection. In the primary kidney transplant group, 202 kidney allografts received FK506 and low-dose steroids as the baseline immunosuppression following kidney transplantation.

In the heart study, patients were also divided into two studies. In one group, 42 patients were treated with FK506 as primary immunosuppression, while in the second group, 10 patients were converted to FK506 because of persistent rejection.

Diagnostic evaluations

For patients who were experiencing organ dysfunction, the final catagorization of dysfunction was based upon clinical, biochemical and/or histopathologic findings. For all patients, either as primary or as rescue therapy, cause(s) of organ dysfunction were carefully sought for, the workup being customized to the organ transplanted. Ultrasonic determination of vessel patency and radiographic 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 FK506 therapy. All biopsies were blinded interpreted by a single experienced liver pathologist (AJD). Biopsy specimens were fixed in neutral buffered formalin and routinely stained with hematoxylin and eosin, trichrome and reticulin stains. The criteria used for pathologic diagnosis have been clearly defined in previous reports (9, 10).

Timing and details of therapy

Initiation of treatment with FK506 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 FK506 was 0.075 to 0.15 mg/kg, given intravenously over a period of 4 hours, although a continuous infusion protocol was initiated in August, 1990. This was continued until the patient was able to ingest the oral form of FK506. Generally, oral dosages of FK506 were given at 0.30 mg/kg/d, given in two divided doses. Dose adjustments of FK506 were based upon monitoring of serum trough levels by ELISA (11) to achieve a 12-h trough level of 1.0 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 plus/minus one standard deviation. Protocol biopsies were obtained after initiation of FK506 therapy.

Results

Liver transplantation

Rescue therapy: In this population of 173 patients, in whom many were critically ill at the time of FK506 conversion, a total of 14 deaths was encountered (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 FK506 conversion. Sepsis was the cause of death in 4 patients. Three patients died of hemorrhagic complications. Three patients died of metastatic carcinoma following transplantation. In 2 patients, retransplantation was not considered an option for the failing liver allograft. One patient was started on FK506 with pathologic findings of late chronic rejection, and died of technical causes during an attempted retransplantation. In 1 death, no cause of death could be determined. This patient died at home and had been off of FK506 for 4 months when she died.

The biochemical response of the liver allografts to FK506 was analyzed by classifying patients either into acute or chronic rejection, depending upon the principal histopathologic 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 FK506 were: 4.68 ± 5.91 mg/dl, 240 ± 431 IU/l, and 292 ± 383 IU/l, respectively. These values fell, by the 6th 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 FK506. For the 116 patients treated for this specific indication, the total bilirubin fell to normal values (pre-FK506, 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-FK506, SGOT/SGPT, 200 ± 175 IU/l/275 ± 223 IU/l; 6 months, SGOT/SGPT, 44 ± 72 IU/l/101 ± 68 IU/l).

In each case where histopathologic changes were predominant, the influence of FK506 on the initial findings of rejection or hepatitis could be evaluated

in serial follow-up biopsies. Overall, 17% of biopsies with a diagnosis of rejection showed worsening of the pathology. 36% of liver biopsies showed no pathologic changes between the pre-FK506 biopsy and the 2-month followup biopsy: 47% of the remaining biopsies showed improvement between the initial and followup biopsies. These changes were particularly impressive in patients whose pretreatment biopsies contained bile duct lesions that generally progress to bile duct disappearance and graft loss, in spite of intensive immunosuppression.

Primary therapy: Ten of the patients have died, leaving an actual survival of 92%. The followup period was between 6 and 12 months. When compared to 325 sequential liver transplants during the preceeding year prior to FK506, the results are stastically significantly better in terms of patient and graft survival, in which the 6-month survival was 79%. This trend was seen in both the adult population (110 patients) and in the pediatric population (15 patients), although the numbers of pediatric patients in the trial were too small to achieve statistical significance. Of the 10 deaths, 5 were due to sepsis, 1 to heart failure, 1 to a cerebral vascular accident, 2 to non-reversible hepatic coma, and 1 to technical complications.

During the followup period, 50% of all recipients were taken off steroids and were maintained on single-drug immunosuppression with FK506. Yet 52.8% of all patients were rejection-free during the entire period of study. The majority of rejection episodes were mild and were easily controlled with a single dose of bolus steroids (either methylprednisolone or hydrocortisone). Only 17.8% of the rejection episodes require further steroid treatment in the form of a steroid taper of additional steroid boluses. In addition, only 11.2% of the patients required. OKT3.

The incidence of serious infections, in spite of the potency of FK506, has not appeared to be alarming. Of note, is that the incidence of cytomegalovirus infections did not appear to be increased, when compared to patients on cyclosporine.

Randomized trial of FK506 versus CyA: A preliminary analysis of a randomized, prospective trial comparing FK506 with CyA in primary liver transplantation appears to verify the lower incidence of rejection and greater ease in treatment of rejection episodes, with less adverse effects. Eighty-one patients were randomized to either cyclosporine (n = 41) or FK506 (n = 40), along with low-dose steroid therapy. The 6-month patient survival was 95% for the FK506-treated group, while the corresponding value for the CyA-treated group was 89%. The corresponding graft survival was 93% and 78%. Two patients in the FK506-treated group and 6 patients in the CyA-treated group were retransplanted. The total percentage of patients in the FK506 group who were rejection-free during the entire length of followup was 53.7%, while that for CyA was 15.2%. The mean days to the first rejection was 21.5 d for the FK506-treated group, and was 9.9 d for the CyA-treated group. OKT3 was used in 30% of CyA-treated patients, while only 20% of FK506-treated patients received OKT3, for treatment of the original allograft: 67.5% of the CyA treated patients were converted to FK506, an average of 20 d after liver transplantation.

Renal function in both groups of patients was assessed by the requirement for hemodialysis and the serum creatinine at monthly determinations. The comparative incidence for hemodialysis requirement between the FK506 and CyA groups was 10% and 21.6%, respectively. Longterm hemodialysis (after 3 months post-transplant) was only required in 1 patient in each group.

The incidence of opportunistic infections was essentially the same for both groups. Patients who were randomized to CyA had a 22.5% incidence of cytomegalovirus infections (CMV). This compared to 22.0% incidence for patients on FK506. In the 13 CyA patients who were not switched to FK506, the incidence of CMV was 23% (3/13), and only 1 of the 3 patients received OKT3. In the FK506 patients, 3 of the total 9 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 CyA-randomized group was 52.9% versus 26.9% for the FK506-treated group ($p < 0.01$), at 3 months post-transplant. The incidence of hypertension in the 14 patients, who were on CyA at the 3-month post-transplant period, was 64.2% (9 of 14 patients).

Kidney transplantation

Rescue therapy: A total of 27 patients were converted from cyclosporine-based immunosuppression to FK506-based immunosuppression for persistent kidney rejection. The median time to FK506 rescue was 2 months (range 1 wk to 63 months). Three deaths were encountered (1 from cardiac arrhythmia, 1 from hypertensive stroke and 1 from disseminated lymphoproliferative disease). Of the 27 patients, 12 grafts were lost (including the 3 deaths). Five had chronic rejection, 1 had severe CyA-related interstitial fibrosis, 1 was a primary nonfunction with rejection, and the last suffered severe humoral rejection.

There were 15 successful rescue attempts. Acute

cellular rejection was the cause of kidney allograft dysfunction in 14/15 of these grafts. The overall serum creatinine prior to FK506 conversion in the successful switches was 5.1 ± 4.4 mg/dl. The average creatinine after FK506 switch was 2.3 ± 1.2 mg/dl. There was a marked ability to utilize small doses of steroids, 3 were off prednisone, 3 were taking 5 mg/d, 2 were taking 7.5 mg/d, 5 were taking 10 mg/d, 1 was taking 15 mg/d, and 1 was taking 20 mg/d.

Primary therapy: FK506 was used from the outset with low doses of steroids to treat 202 primary kidney grafts. This was compared to a group of 180 kidney allografts treated with CyA during the same period of time. Of the total 382 renal allografts transplanted, all but 37 were cadaveric renal allografts: 27% of the recipients were undergoing kidney retransplantation: 19% of the kidney allografts were pediatric *en bloc* kidney allografts.

The actuarial patient survival following kidney transplantation was 96% for all patients; for the FK506 and CyA groups, the figures were 95% and 97%, respectively. The corresponding 1-yr graft survival was 83% and 85% for the FK506 and CyA groups, respectively. In the patients who were undergoing their first kidney transplant, the 1-yr graft survival was 85% and 86%, while the 1-yr graft survival in patients undergoing retransplantation was 77% and 81%, for FK506 and CyA. The percentage of patients who experienced rejection was 57% for the FK506 group and 54% for the CyA group. The incidence of steroid-resistant rejection, which was reflected by the requirement for OKT3 use, was 21% for FK506-treated patients and 38% for CyA kidney patients. The mean serum creatinine and blood nitrogen was the same for both groups.

Heart transplantation

Rescue therapy: Ten patients were converted from cyclosporine to FK506 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 (14), included mononuclear cell infiltration, arteritis and in some instances, interstitial fibrosis. All patients had failed conventional immunotherapy, including at least two courses of anti-lymphocyte preparations, and two courses of augmented steroids during the preceding 6 months. The grading of endomyocardial biopsies, prior to conversion to FK506, was 2.70 ± 0.48. Using the same criteria, the mean value of the followup biopsies after FK506 was graded at 0.70 ± 0.67 ($p < 0.01$). The mean prednisone dose prior to FK506 conversion was 14 mg/d, after FK506 conversion this fell to 5.5 mg/d. Only 1 death occurred during the period of followup in a patient with disseminated aspergillosis.

Primary therapy: Forty-two patients received FK506 from the outset following heart transplantation. Twenty-seven patients were on circulatory assist devices prior to heart transplantation. The mean followup was 40 d. Four patients have died, with an actual patient and graft survival of 93%. One patient with known pulmonary hypertension died on the 3rd post-transplant d from right heart failure. One patient, with preexisting lung disease and bronchietasis, died from pulmonary infection, while 2 other patients died sudden deaths, without a known cause of death.

The rejection-free rate within the first 90 d was 65%. Only 1 patient required OKT3. Heart function was excellent in all patients. The average left ventricular ejection fraction, determined by gated nuclear scans of echocardiography, was 70% (range 58% to 75%). Hypertension, which was seen in 70% of CyA-treated heart transplant recipients, was only 15% in the heart transplant patients treated with FK506.

Limitations

Adverse reactions requiring treatment or adjustment of FK506 doses can be catagorized into four primary areas. These are: 1) alterations in kidney function, 2) alterations in glucose metabolism, 3) neurotoxicity, and 4) 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. Hyperkalemia is seen in 35% of patients following administration of FK506. Treatment of hyperkalemia is generally with potassium-binding resins and potassium-restricted diets. Addition of a synthetic mineralocorticoid, Florinef, relieves the hyperkalemia by increasing potassium excretion by the kidney. Decrement in renal blood flow has 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: perioperative hypotension, use of nephrotoxic antibiotics, and degree of preexisting renal dysfunction. The alteration in renal function seen in patients on FK506 is similar to that seen in patients on cyclosporine. These changes are responsive to reduction in doses of FK506. The incidence of renal failure requiring chronic hemodialysis is on the order of 4%, based on studies of liver transplant patients, although no patients have required maintenance hemodialysis 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 hyperglycemia. The incidence of new-onset diabetes, i.e., those patients requiring insulin, is approximately 15% in FK506 transplant patients. The incidence of new-onset diabetes in other immunosuppressive regimens, incorporating cyclosporine, 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 FK506 administration. Expressive aphasia has been seen in 4 liver transplant patients, although this has not been seen in any other types of FK506-treated patients. New-onset seizures have also been reported in liver transplant patients, especially during the perioperative 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 FK506.

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 tumor. PTLD has been associated with all types of immunosuppressive therapy. The incidence of PTLD in the cyclosporine era is generally estimated between 2% and 4%. The median time following transplantation to the development of PTLD is 6 months, while the majority of these tumors occur withlin 12 months following transplantation.

A total of 16 patients have developed *de novo* PTLD lesions, while on FK506 therapy. Seven of these patients died, although PTLD was associated with death in only 5 deaths. The remaining 9 patients had relatively mild forms of PTLD, 3 of these had a mononucleosis syndrome, with presentation of sore throat and tonsillar enlargement. Treatment with lower immunosuppression and intravenous acyclovir cured all of them. In the remaining 6 patients, 3 required operative procedures (2 small bowel resections, 1 liver resection) which were directly related to PTLD, while the other 3 were treated by reduction of immunosuppression only. The incidence of *de novo* PTLD following initiation of FK506 therapy is 1.4%. All of the cases of PTLD occurred within the 1st yr following initiation of FK506, with the median time from FK506 therapy to onset of disease being 4 months. FK506 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 FK506 for non-transplanted 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 FK506-treated transplant patients is 20%. This figure is similar to that seen in transplanted patients on cyclosporine. No patients treated with FK506 for non-transplant indications have developed CMV infections.

Discussion

Cyclosporine-based immunosuppression significantly enhanced both patient and graft survival in all solid organ transplants, when compared to the era of azathioprine and steroids (12). The most common complicating factor has been the development of rejection, occurring in over 60% of all cyclosporine-treated patients. In addition, the sequela of overimmunosuppression in attempts to treat rejection, such as use of excessive steroids, anti-lymphocyte 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, and easier treatment of rejection, would decrease both graft and patient loss. From the results of our preliminary studies presented here, the use of FK506 in liver transplantation has these advantages. FK506 appears to not only decrease the absolute incidence of rejection episodes, and allows for marked reduction in steroid doses, but makes the treatment of rejection much simpler.

The ability of a new immunosuppressive agent to be dose-adjustable for treatment of acute and chronic rejection would represent an important asset, which has only been ascribed to steroids in the past. FK506 can be used in this manner. In fact, the first response to a developing rejection is to increase the dose of baseline FK506. In rescue therapy, the marked ability of FK506 to reverse acute rejection in both kidney and heart rejection, and both acute and chronic rejection in liver transplantation, has not been seen with any immunosuppressive agent in the past. While the mechanism by which FK506 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 mechanisms of action of FK506 and CyA are not clearly delineated. Both drugs bind to proteins having a peptidyl-prolyl cis-trans isomerase activity (13). They also share some of the same side-effects, although they differ significantly in other important respects. The incidence of side-effects of the two drugs is similar. Neurotoxicity is manifested by insomnia, mild tremors, headaches, photophobia and hyperesthesias. Gastrointestinal symptoms include diarrhea, anorexia, flatulence and gas bloating. One of the major benefits of FK506 appears to be a relative lack of some of the side-effects of cyclosporine. Some of these are cosmetic, such as hirsuitism and gingival hyperplasia, which has not been seen with FK506. Other more significant side-effects, such as hypertension, appear less in the FK506 patients than those on cyclosporine. Hypertension, which is seen in 60–70% of all cyclosporine-treated patients, may result in complications such as hypertensive stroke, cardiomyopathy, and augment renal failure. The incidence of hypertension in FK506-treated patients appears to be at least 50% less. The ability to use less steroids in patients with FK506, when compared to cyclosporine, may result in less complications ascribed to chronic steroid use, such as: osteoporosis, Cushinoid habitus, stunted growth, ulcerogenesis, diabetes.

Prospective, randomized trials comparing FK506 therapy with cyclosporine-based immunosuppression are currently underway. These studies will help identify areas in which FK506 may be more advantageous, or more disadvantageous, than present-day immunosuppression. The initial preliminary results carried out in liver transplantation, at this center are encouraging. The results at other centers will hopefully confirm these findings.

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