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Overview of FK506 in Transplantation

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In 1987, Ochiai and coworkers reported on the qualities of a new immunosuppressive agent, FK506, isolated from the fermentation broth of a soil fungus. Streptomyces tsukubaensis (1) Extensive in vitro studies demonstrated its effectiveness in suppressing mixed lymphocyte cultures, presumably by inhibiting IL2 synthesis following alloactivation (2). The receptor for FK506 has been identified and characterized as a peptidyl-prolyl cis-trans isomerase (3).

The background for the clinical development of FK506 has been well documented by Starzl (4). In vivo studies using a number of animal models have shown a marked ability to prevent rejection following various types of organ transplants (5–7), as well as to prevent the development of graft-versus-host disease (GvHD) following bone marrow transplantation (8). More interestingly, FK506 possesses the ability to reverse ongoing rejection in animal models (7,9), as well as established GvHD (10). This characteristic is unique, for it is well known that cyclosporine (CsA) will not reverse established ongoing alloimmune responses. These properties continue to be evident during the clinical testing of FK506. We will attempt to summarize the results of all human transplantation models in which FK506 has been utilized as either "rescue" therapy and/or "primary" therapy, including liver (11,12), kidney (13), heart (14), and bone marrow transplantation (11), 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 FK506 as part of 3 studies; a) rescue, in which 57 patients were entered for the diagnosis of acute rejection and 116 were converted from CsA to FK506 for chronic rejection; b) 110 primary liver transplant recipients who were treated with FK506 and low-dose steroids as the baseline immunosuppression following liver transplantation; and c) a subsequent study involved a total of 81 patients, prospectively randomized to either FK506 or CsA as the baseline immunosuppression following liver transplantation.

In the kidney study, patients were treated with FK506 as part of 2 studies; a) rescue, in which 21 patients were entered for the diagnosis of rejection; and b) 65 primary kidney

transplant recipients who were treated with FK506 and low-dose steroids as the baseline immunosuppression following transplantation.

In the heart study, patients were also divided into 2 groups; a) 30 patients were treated with FK506 as primary immunosuppression; and b) 10 patients were converted to FK506 because of persistent rejection.

In the bone marrow study, 11 patients were entered as part of a rescue study and treated with FK506 for evidence of persistent manifestations of GvHD 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 histopathologic findings. For all patients, either as primary or as rescue therapy, cause(s) of organ dysfunction were carefully looked for the workup being customized to the organ or tissue transplanted. Ultrasonic determination of vessel patency and radiographic evaluation of the biliary or urinary systems were used to rule out technical or mechanical defects. 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 and interpreted by a single experienced transplant 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 (15,16).

Timing and Details of Therapy

Initiation of FK506 treatment was done in the hospital and given initially as a parenteral dose (0.075–0.15 mg/kg, IV), continued until the patient was able to ingest the oral form of FK506, generally at 0.3 mg/kg/day, in 2 divided doses. Dose adjustments of FK506 were based upon monitoring serum trough levels by ELISA (17) to achieve a 12-hour trough level between 1–2 ng/ml and also according to clinical or biochemical parameters.

Evaluation of Response

Periodic determinations of liver and kidney functions were performed, including total bilirubin, serum glutamic transaminases, SGOT and SGPT, alkaline phosphatase, blood urea nitrogen, and serum creatinine (SCr). All values are expressed as the value plus/minus 1 standard deviation. Protocol biopsies were obtained after initiation of FK506 therapy.

RESULTS

Liver Transplantation

Rescue Therapy—In this population of 173 patients, many of whom were critically ill at the time of FK506 conversion, a total of 14 deaths were 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; 3 died of hemorrhagic complications; and 3 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 pathological findings of late chronic rejection and died of technical causes during an attempted retransplantation. In 1 case, no cause of death could be determined; the patient died at home and FK506 had been discontinued 4 months earlier. The biochemical response of the liver allografts to FK506 was analyzed by classifying patients into either acute or chronic rejection, depending upon

the principal histopathologic findings. Prior to FK506 therapy, the TBIL, SGOT, and SGPT values for the 57 patients who were treated for acute rejection, documented on liver biopsy or as judged by biochemical and clinical parameters were; 4.68±5.91 mg/dl, 240±431 IU/I, and 292±383 IU/I, respectively. These values fell by the sixth month to: 0.76±1.41 mg/dl, 98±163 IU/I, and 90±128 IU/I, respectively. Patients with a presenting 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) whereas the average transaminase values were still slightly elevated above normal values (pre-FK506, SGOT/SGPT, 200±175 IU/I/275±223 IU/I; 6 months, SGOT/SGPT, 44±72 IU/I/101±68 IU/I).

A clinicopathologic study of the results of conversion of liver allografts from CsA to FK506 immunosuppression revealed that the biochemical improvement seen above was correlated with histopathologic improvement (18). The biochemical improvement in both acute and chronic rejection occurred earlier and in greater proportion than the pathologic findings. Patients with acute rejection fared better than those with chronic rejection, with a higher response rate. In patients with chronic rejection, liver function studies and the degree of bile ductular injury were signficantly worse among those who failed than those who responded.

Primary Therapy—Eight patients died, leaving an actual survival of 92.7%. The follow-up period was between 6 and 12 months. When compared to 325 sequential liver transplants during the preceding year (prior to FK506), the patient and graft survival results were statistically significantly better: 6-month survival was 79%. Of the 8 deaths, 5 were due to sepsis, 1 to heart failure, 1 to a cerebral vascular accident, and 1 to nonreversible hepatic coma. During the follow-up period, 50% of all recipients were taken off steroids and maintained on single-drug immunosuppression with FK506. Yet 52.8% of all patients were rejection free during the entire study period. 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 OKT3. The incidence of serious infections, in spite of FK506 potency, has not appeared to be alarming. The incidence of serious infections was about 50% less than seen with an historical group of patients given CsA. It is noteworthy 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 primary FK506 series, a prospective, randomized study was initiated in patients undergoing primary liver transplantation, which incorporated FK506 and CsA, along with steroids. After liver transplantation, 81 recipients were randomized to either FK506 (41 patients) or CsA (40 patients). A single bolus of 1 gm of methylprednisolone followed by a daily dose of 20 mg of methylprednisolone was the baseline steroid therapy for both groups. Biochemical and histopathologic parameters were monitored to determine the effectiveness of each therapy in preventing rejection. Rejection episodes were treated with a single bolus of 1 gm of methylprednisolone. If this treatment failed to reverse the rejection episode, a total of 50 mg of OKT3 was administered. The CsA patients who failed to respond to therapy were converted to FK506, in attempts to rescue the dysfunctional grafts. With the ability to rescue CsA-randomized dysfunctional grafts with FK506, patient and graft survival were essentially the same. Figure 1 shows the patient survival curves for the 2 treatment groups. The 3-month patient survival for the 2 groups was 100% for the FK506 patients and 95.6% for the CsA group. The 6-month figures for patient survival were 94.7% and 88.8%, respectively. Figure 2 demonstrates the corresponding graft survival curves for these

patients. Three-month graft survival was 93.4% for the FK506 group versus 84.8% for the CsA group. The corresponding 6-month figures were 93.4% and 78.1%.

One measure of the effectiveness of a baseline immunosuppressive regimen is the rejection-free rate following transplantation. Since liver allograft rejection was strictly defined biochemically, histologically, and/or clinically, this parameter was a simple, objective endpoint. Figure 3 shows the percentage of patients in each group who remained rejection-free. In both groups, the major incidence of rejection episodes occurred within the first 30 days: a mean day of 21.5 in the FK506 group, for the first rejection contrasted with a mean of 9.9 days in the CsA group ($p<0.005$). In addition, a statistically significantly larger number of patients in the FK506 group remained rejection free during the follow-up period ($p<0.025$). At 1 month, the rejection-free rate for the FK506 patients was 61%, while the CsA value was 18.1% ($p<0.001$). There were few late rejections; 3 in the FK506 group over the next 6 months, with 1 additional late rejection in the CsA group.

These results are in agreement with the original primary therapy group when compared with the historical CsA experience. The major benefit of a prospective randomized trial is to evaluate other surrogate parameters. The need for insulin therapy and the infectious disease profiles between the 2 groups were essentially the same. Renal function in both groups was assessed by the requirement for hemodialysis and the monthly SCr determinations.

Hemodialysis was initiated in 6 CsA patients while still on CsA, whereas 3 other CsA patients required hemodialysis during the conversion period to FK506. In the FK506-randomized group, 4 patients were dialyzed during the posttransplant period. The comparative incidence for hemodialysis requirement between the FK506 and CsA groups (excluding terminal hemodialysis) was 10% and 21.6%, respectively. Long-term hemodialysis (after 3 months posttransplant) was only required in 1 patient in each group. During the first 4 months, there was little appreciable difference in the SCr for the 3 groups with functioning kidneys. 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 FK506-treated group ($p<0.01$), at 3 months posttransplant. The incidence of hypertension in the 14 patients who were on CsA at 3 months posttransplant was 64.2% (9 of 14 patients).

Kidney Transplantation

Rescue Therapy—A total of 21 patients were converted from CsA-based immunosuppression to FK506-based immunosuppression for persistent kidney rejection. No deaths were encountered. Of the 21 patients, 10 were classified as late rejection episodes (>60 days), while 11 were treated early in the posttransplant course (< 60 days). Seven of the 11 early rescues were successful, in contrast to only 4 of 10 late rescues. Most of the FK506 failures were in patients who had chronic glomerulosclerosis and chronic rejection on biopsy, prior to FK506 rescue. The results were better in those patients with acute cellular rejection. The SCr at conversion time was also correlated with successful therapy. Four of 5 (80%) patients with a SCr <3 mg/dl had good renal function, while only 7 of 16 (44%) patients with a preconversion SCr >3 mg/dl had a functioning kidney. The overall SCr prior to FK506 conversion in the 11 successful conversions was 3.70±2.15 mg/dl, excluding the SCr of 4 patients who were on dialysis during FK506 conversion. The average creatinine after the FK506 switch was 2.84±1.40 mg/dl, with all 11 grafts functioning.

Primary Therapy—FK506 was used from the outset with low-dose steroids to treat 65 recipients of primary kidney grafts. Of the 66 renal allografts transplanted, all but 2 were cadaveric renal allografts: 35% of the 65 recipients were regrafts: 46% of the patients were classified as highly sensitized, with a panel reactive antibody (PRA) level >40%. Nine

percent underwent renal transplantation in the face of a positive cytotoxic crossmatch using current or historical serum samples. Sixteen of the 66 grafts were pediatric en bloc kidney allografts.

The actual patient survival posttransplantation was 98%. One diabetic patient with preexisting coronary artery disease died from a postoperative myocardial infarction 3 days posttransplantation. The corresponding overall graft survival was 79%. In the patients with PRA <40%, graft survival was 83%, and in those with PRA >40% it was only 73%. Fifty-six percent of patients have remained rejection free. The treatment for rejection episodes was generally accomplished with steroid boluses. As a reflection of the ease by which rejection was managed, only 29% of kidney patients required OKT3.

Heart Transplantation

Rescue Therapy—Ten patients were converted from CsA to FK506 between 3 and 50 months posttransplant. Persistent heart rejection defined by a >2+ grading of the endomyocardial biopsy by the Billingham criteria (16), included mononuclear cell infiltration, arteritis, and in some instances, interstitial fibrosis. All patients had failed conventional immunotherapy, including at least 2 courses of antilymphocyte preparations and 2 courses of augmented steroids during the preceding 6 months. The grading of endomyocardial biopsies, prior to conversion to FK506, was 2.7±0.48. Using the same criteria, the mean value of the follow-up biopsies after FK506 was graded at 0.7±0.67 ($p<0.01$). The mean prednisone dose prior to FK506 conversion was 14 mg/day, and after FK506 conversion this fell to 5.5 mg/day. Only 1 death occurred during follow-up in a patient with disseminated aspergillosis.

Primary Therapy—Following heart transplantation 30 patients received FK506 from the outset. Eight patients were on circulatory assist devices prior to heart transplantation. Follow-up ranged from 1–10 months. Four patients died, with an actual patient and graft survival of 87%. One patient with known pulmonary hypertension died on the third posttransplant day from right heart failure. One patient with preexisting lung disease and bronchietasis died from pulmonary infection, while 2 others died suddenly without a known cause. The rejection-free rate within the first 90 days was 60%. Only 1 patient required OKT3. Heart function was excellent in all patients. The average left ventricular ejection fraction, determined by gated nuclear scans or echocardiography, was 70% (range 58–75%).

Bone Marrow Transplantation

Rescue Therapy—Eleven patients with manifestations of chronic GvHD following bone marrow transplantation were placed on FK506. Six patients had an original diagnosis of chronic myelogenous leukemia, 4 were given bone marrow transplants for acute lymphoblastic leukemias, 1 had aplastic anemia. All grafts were taken from HLA-identical siblings. All patients were on or had been on high doses of CsA and steroids. Table 1 shows the organ systems involved. The most common sites of involvement were skin and liver, followed by lung, GI, and musculoskeletal. The most objective parameters to evaluate response to FK506 have been those with liver and skin involvement. The mean time after bone marrow transplantation to the time of FK506 therapy was 17.7 months, and the mean follow-up was 3.8 months.

Of the 9 patients with liver involvement, 5 were referred for consideration for liver transplantation. Two of these eventually required liver transplantation, but both died; 1 from sepsis following liver transplantation, the other failed to awaken after transplantation having been in Stage IV coma prior to transplantation. One other patient died of unknown causes at home, and the other 6 patients had a marked response to FK506 rescue therapy. In the 10

patients with skin involvement, 7 improved, while 3 with scleroderma-like involvement had stable skin lesions. Two patients with moderate to severe obliterative bronchiolitis died from worsening lung disease. The remaining organ system involvement of musculoskeletal and GI tract has not shown progression during FK506 therapy.

DISCUSSION

CsA-based immunosuppression significantly enhanced both patient and graft survival in all solid organ transplants, when compared to the era of Aza and steroids (19). Its use in bone marrow transplantation has decreased the incidence and severity of GvHD (20). Nevertheless, most centers experience an unacceptably high complication rate related to ongoing GvHD or rejection. These immunologically related complications occur in over 70% of all CsA-treated patients. In addition, the sequelae of over-immunosuppression in attempts to treat rejection or GvHD, such as excessive steroids or antilymphocyte preparations, are fraught with a high incidence of infectious complications. Logically, 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, FK506 treatment in transplantation has these advantages. FK506 appears not only to decrease the absolute incidence of rejection episodes, but makes rejection treatment much 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, which in the past has only been ascribed to steroids, 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 kidney and heart rejection, acute and chronic rejection in liver transplantation, and chronic GvHD in bone marrow transplantation, has not been seen with any other immunosuppressive agent. Whereas the mechanism by which FK506 is able to accomplish this is unknown, it appears that it would include mechanisms other than simply inhibition of IL-2 synthesis.

Prospective, randomized trials comparing FK506 therapy with CsA-based immunosuppression are currently underway. The preliminary results in liver transplantation at the University of Pittsburgh are encouraging. Multicenter 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 suggest that a conversion to FK506 will allow endangered allografts to be salvaged. Other randomized trials in kindey, heart, and bone marrow transplantation will await the results of the liver trials.

SUMMARY

FK506 is a potent immunosuppressive agent which is undergoing clinical testing in liver, kidney, heart, and bone marrow transplantation. It has been shown to effectively prevent and reverse ongoing rejection in these models. From the outset, FK506 was used with low-dose steroids to treat 110 primary liver, 30 heart, and 66 kidney graft recipients. FK506 was also used in the setting of complications related to CsA or to ongoing chronic or acute rejection. One hundred seventy-three liver, 21 kidney, 10 heart, and 11 bone marrow recipients were converted to FK506 and low-dose steroids, from a combination of CsA, steroids, and/or Aza. A randomized, prospective trial comparing FK506 with CsA in primary liver transplantation has verified the lower incidence of rejection and greater ease in treating rejection episodes, with fewer adverse effects. In summary, FK506 has proven to be an effective baseline immunosuppressive agent, as well as a dose-adjustable agent for the treatment of rejection.

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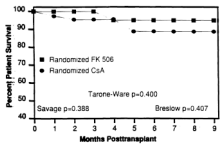

Figure 1.

Patient survival curves for all FK506- and CsA-randomized patients are shown. The 3-month patient survival for the 2 groups was 100% for the FK506 patients and 95.6% for the CsA group. The 6-month patient survival rates were 94.7% and 88.8%, respectively.

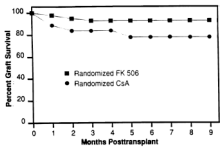

Figure 2.

The corresponding graft survival curves for the CsA- and FK506-randomized patients are shown. The 3-month graft survival for the 2 groups was 93.4% for the FK506 versus 84.8% for the CsA group. The corresponding 6-month figures were 93.4% and 78.1%.

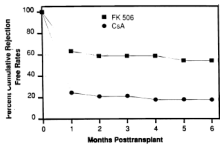

Figure 3.
Relative frequency of patients who are free of rejection is plotted against time following liver transplant.

Table 1

GvHD following bone marrow transplantation treated with FK 506 (n=11).

Organ Affected	Patients Affected	Response		
		Better	Same	Worse
Skin	10	7	3	0
Liver	9	7	0	2[a]
Lung	5	1	2	2[b]
GI	5	1	4	0
Musculoskeletal	3	0	3	0

[a] 2 patients required liver transplantation, but died following transplantation.

[b] 1 patient died following liver transplantation with GvHD of lung, 1 died from respiratory failure.