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Current status on FK506 in organ transplantation

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I. INTRODUCTION

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

The background for the clinical development of FK506 was well documented by Starzl in the opening remarks of a satellite symposium on FK506, held in Barcelona, Spain in November, 1989, as part of the European Society of Organ Transplantation (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). More interestingly, FK506 possess the ability to reverse ongoing rejection in animal models (7-8). This characteristic is unique, since it is well known that cyclosporine will not reverse established ongoing rejection. These properties served as the initiative to pursue clinical testing of FK506.

The objective of this manuscript is to update the reader on the current status of the use of FK506 in human organ transplantation, at the

University of Pittsburgh. We will attempt to summarize the results of "rescue" therapy and "primary" therapy with FK506 in human liver (9,10), kidney (11) and heart (12) transplantation.

II. METHODS

1. 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.

2. 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 21 patients were entered

for the diagnosis of rejection. In the primary kidney transplant group, 65 patients were treated with 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, 30 patients were treated with FK506 as primary immunosuppression, while in the second group, 10 patients were converted to FK506 because of persistent rejection.

3. 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 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 FK 506 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 (13,14).

4. 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 FK506 was 0.075 to 0.15 mg/kg, given intravenously over a period of four hours. 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 (15) to achieve a 12 hour trough level of 1.0 ng/ml, and also by adjustment according to clinical or biochemical parameters.

5. 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.

III. RESULTS

1. Liver Transplantation

a) 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 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. Three patients died of hemorrhagic 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 pathologic findings of late chronic rejection, and died of technical causes during an attempted retransplantation. In one case, no cause of death could be determined. This patient died at home and had been off of FK506 for four months when she died.

The biochemical response of the liver allografts to FK506 was analyzed by classifying patients either into acute or chronic rejection, dependent upon the principle 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 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 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 followup 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 two 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.

b) 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 preceding year prior to FK506, the results are statistically 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 ten deaths, five were due to sepsis, one to heart failure, one to a cerebral vascular accident, two to non-reversible hepatic coma, and one 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 both steroids (either methylprednisolone or hydrocortisone). Only 17.8% of the rejection episodes require further steroid treatment in the form of a steroid taper or additional steroid boluses. In addition, only 11.2% of the patients require OKT3.

The incidence of serious infections, in spite of the potency of FK506, has not appeared to be alarming. The incidence of serious infection was about 50% less than seen with a historical group of patients given cyclosporine. Of note, it did not appear to be increased, when compared to patients on cyclosporine.

2. Kidney Transplantation

a) Rescue Therapy

A total of 21 patients were converted from cyclosporine based immunosuppression to FK506 based immunosuppression for persistent kidney rejection. No deaths were encountered. Of the 21 patients, 10 were classified into late rejection episodes (>60 days), while 11 were treated early in the post-transplant course (<60 days). 7 of the 11 early rescues were successful, in contrast to only 4 of 10 late rescues. Most of the failures of FK506 rescue therapy in this group of patients were in patients who had chronic glomerulosclerosis and chronic rejection on biopsy, prior to FK506 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. 4 of 5 (80%) patients with a serum creatinine <3.0 mg/dl have good renal function, while only 7 of 16 (44%) patients with a pre-conversion serum creatinine >3.0 mg/dl have a functioning kidney. The overall serum creatinine prior to FK506 conversion in the 11 successful switches was 3.70 ± 2.15 mg/dl, excluding the serum creatinine values of 4 patients who were on dialysis at the time of FK506 conversion. The average creatinine after FK506

switch was 2.84 ± 1.40 mg/dl, with all 11 grafts functioning.

b) Primary Therapy

FK506 was used from the outset with low doses of steroids to treat 65 recipients of primary kidney grafts. Of the total 66 renal allografts transplanted, all but 2, were cadaveric renal allografts. 35% of the 65 recipients were undergoing kidney retransplantation. 46% of the patients were classified as highly sensitized, with a panel reactive antibody (PRA) level >40%. 9% of the patients underwent renal transplantation in the face of a positive cytotoxic crossmatch using current or historical serum samples. 16 pediatric *en bloc* kidney allografts of the 66 allografts were used.

The actual patient survival following kidney transplantation was 98%. One patient died from a post-operative myocardial infarction, 3 days following transplantation, in a diabetic patient with preexisting coronary artery disease. The corresponding overall graft survival was 79%. In the patients who had a PRA <40%, the graft survival was 83%, while the graft survival in patients with PRA >40% was only 73%. The percentage of patients who have remained rejection free was 56%. The treatment for rejection episodes were generally accomplished with steroid boluses. As a reflection of the ease by which rejection was managed, the requirement for OKT3 use was 29% for kidney patients.

3. Heart Transplantation

a) 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 six 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 one death occurred during the period of followup in a patient with disseminated aspergillosis.

b) Primary Therapy

Thirty patients received FK506 from the outset following heart transplantation. 8 patients were on circulatory assist devices prior to heart transplantation. Followup 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 preexisting lung disease and bronchiectasis, died from pulmonary infection, while two other patients died of sudden deaths, without a known cause of death. 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 ejection fraction, determined by gated nuclear scans or echocardiography, was 70% (range 58% to 75%).

IV. 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 (16). Nevertheless, most centers experience an unacceptably high graft loss and patient losses are high in liver and heart transplantation. The most common complicating factor has been the development of rejection, occurring in over 70% of all cyclosporine treated patients. In addition, the sequelae 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 current situation with the Food and Drug Administration (FDA) regarding the use of FK506, is that it will require further substantiation of the efficacy of FK506 in multicenter trials. 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 current day immunosuppression. The initial studies will be carried out in liver transplantation, followed by studies in kidney transplantation. Heart transplantation and possibly bone marrow transplantation, will await the results of the initial liver and kidney multicenter trials. A well defined endpoint, other than patient or graft loss should be utilized, since the data presented here also suggests that a conversion to FK506 will allow for allografts in danger of being lost to rejection to be salvaged. Future trials examining the role of FK506 in solid organ allograft rejection are being devised, either separately from the primary trials, or perhaps, as part of the primary trials.

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