USE OF FK506 IN THE TREATMENT OF LIVER ALLOGRAFT REJECTION

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Abstract. *FK506 was used in 71 liver transplant recipients who, while on cyclosporine based immunosuppression, were suffering from chronic or acute rejection. When the biochemical and histopathologic parameters for response to FK506 conversion were studied, it was evident that the subsets of patients with acute and chronic rejection sustained a statistically significant improvement in liver function. Those patients with coexisting hepatitis involving the liver allograft, when converted to FK506, had some improvement in biochemical parameters. However, histopathologically, 35 percent of these livers showed progressive involvement with hepatitis.*

Introduction

Cyclosporine has been a major advance in the armamentarium of immunosuppressive agents used in clinical transplantation. The use of cyclosporine and steroids have increased patient and graft survival in liver transplantation.[1, 2] Cyclosporine has been shown to inhibit interleukin-2 synthesis and therefore inhibit T cell proliferation. Nevertheless, clinical rejection occurs in over 60 percent of all liver transplant recipients. Rejection continues to be a common cause of retransplantation, and death is often a sequelae of treatment of rejection. A number of adverse effects of cyclosporine have been well defined: nephrotoxicity, hypertension, neurotoxicity and hirsutism. Also, a number of less well defined side effects have been described. Alterations in clinical immunosuppression to prevent or reverse these and other side effects have included: 1) reduction of cyclosporine dose, or 2) addition of azathoprine, antilymphocyte antibodies (ALG) or other agents with concomitant reductions in the cyclosporine dose. These methodologies have their inherent dangers, i.e., increasing susceptibility to rejection and infection, respectively.

FK506 is a potent and novel immunosuppressive agent. The initial use of FK506 was for conversion of liver transplant patients considered to have failed cyclosporine therapy and was termed "rescue therapy". Our first report included 40 patients who received FK506 because they were rejecting their liver grafts in spite of conventional immunosuppression.[3] The initial protocol combined low doses of FK506 with cyclosporine, however, this combination was accompanied by a number of adverse reactions. Eventually, it was learned that both drugs compete for metabolism via similar pathways so that a clean conversion was subsequently made for patients who were undergoing rescue therapy.

A larger series of patients, which included 246 patients, was reported at the XIII International Congress of the Transplantation Society. We confirmed the beneficial biochemical response of failing liver allografts under cyclosporine therapy to FK506 rescue therapy.[4] Patients with acute rejection and to a lesser extent, chronic rejection, responded well to FK506 conversion.

The following is a summary of clinicopathologic analysis of cyclosporine treated liver transplant recipients with either chronic or acute rejection. These patients were converted from cyclosporine, steroids and/or azathioprine to FK506 with or without low dose steroids.

Patients and Methods

Study Design. This trial was conducted at the University of Pittsburgh, Presbyterian University Hospital, Children's Hospital 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. The accrual period for this study began on February 28 1989 and continued to December 31 1989. A minimum followup

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period of nine months was obtained on all patients.

Patient Profiles. Seventy-one patients were selected for study during this period of time. Most were bearing their first liver allograft at the time of the conversion from cyclosporine to FK506. A smaller number of patients had previously received more than one previous liver transplant, e.g., one patient was carrying his fifth liver transplant at the time of FK506 conversion.

The median time from transplantation to FK506 conversion was 21 months, range 0.1 to 78 months. The median age was 42 years, range 1 to 66 years of age. The sex distribution was slightly male predominant.

Indications. The 71 patients in the study suffered from a wide variety of hepatic disorders requiring transplantation. Most had cryptogenic cirrhosis or post-necrotic cirrhosis due to non A-non B hepatitis (27 percent). Cholestatic cirrhosis (27 percent) and alcoholic cirrhosis (18 percent) made up the other large categories.

All patients switched to FK506-based immunosuppression were selected for this study based on: 1) biochemical graft dysfunction defined by elevated liver function tests (>50 percent) over the lowest value in the prior week and unresponsiveness to standard augmented immunosuppression; 2) no evidence of mechanical dysfunction; 3) lack of significant concomitant infections; 4) adequate pathologic documentation, both prior to and after FK506 conversion. Prior to conversion to FK506, maintenance immunosuppression in all patients consisted of cyclosporine and prednisone, with or without azathioprine. Cyclosporine doses had been maximized to tolerable levels, as limited by renal dysfunction or hypertension. Seventy percent of the patients had received at least one course of OKT3 prior to conversion to FK506.

The indications for FK506 conversion are shown in Table 1. In several instances, more than one coexistent indication was present. Rejection was the indication for treatment in all 71 patients. Acute rejection was seen in 18 allografts. Chronic rejection was seen in 33 allografts. Coexistent hepatitis and chronic rejection was seen in another 20 patients.

Pathologic evaluations. Biopsies were taken at the initiation of FK506 therapy and within two months following FK506 conversion. All liver biopsies were reviewed by a single pathologist (AJD). Biopsy specimens were fixed in neutral buffered formalin and routinely stained with hematoxylin and eosin, trichrome and reticulin stains.

The following histologic criteria were used for the pathologic diagnosis of acute hepatic rejection:

1. A portal tract inflammatory infiltrate consisting of 50-60 percent mononuclear cells intermixed with polymorphonuclear cells and eosinophils.

2. Characteristic localization of the inflammatory cells around and beneath the swollen endothelium of portal capillaries and small veins with infiltration and damage of the epithelium of small bile ductules. The number of damaged bile ductules and the degree of damage generally increased with time. The most severe changes, with ductular loss, were seen in chronic rejection.

3. Absence of histologic findings suggestive of hepatitis.

A diagnosis of chronic hepatic rejection was made if there was evidence of:

1. The obliterative arteriolar lesions that have been found in liver as well as other solid organs.[1,5]

2. Loss of intrahepatic bile ducts, often with only a mild peri- and intraductal chronic inflammatory infiltrate.

3. Portal fibrosis, especially if linkage had occurred between portal tracts or central veins.

4. Absence of lobular changes suggesting hepatitis.

An attempt was made to quantify changes associated with "chronic" rejection. A diagnosis of "early" chronic rejection was made when there was evidence of lymphocytic bile duct damage in <50 percent of portal triads and bile duct loss limited to <25 percent of triads without cholestatic or hepatitic changes. "Late" chronic rejection was defined by duct loss in >50 percent of portal triads, with lymphocytic bile duct damage in remaining triads and with hepatocanalicular cholestasis.

TABLE 1.
INDICATIONS AND STATUS OF FK506 RESCUE ATTEMPTS

Indication for Rescue	*# Functioning*	*# Failed*	*# Died*	*Total*
Acute Rejection	14	1	3	18
Chronic Rejection	22	11	0	33
Chronic Rejection with Hepatitis	16	4	0	20

A diagnosis of hepatitis was made if there was evidence of:

1. Significant panlobular inflammation, piecemeal necrosis, cholangiolar proliferation, disarray with ballooning and spotty individual hepatocyte necrosis and prominent lymphohistiocytic infiltration of the hepatic lobule with inflammatory cell destruction of hepatocytes. The foregoing are not prominent features of rejection under immunosuppression and suggest a diagnosis of viral hepatitis.

2. Positive staining for viral proteins or positive growth of virus from liver biopsy specimens.

Timing and details of therapy: Cyclosporine was discontinued 24 hours prior to initiation of FK506 therapy. Initiation of treatment with FK506 was done in the hospital and was given initially as a parenteral dose with conversion to an oral dose. The inital parenteral dose of FK506 was 0.075 to 0.15 mg/kg given intravenously over a period of four hours. Generally, oral dosages of FK506 were given at 0.30 mg/kg in divided doses. Dose adjustments of FK506 were based upon monitoring of serum trough levels by ELISA[9] and also by adjustment according to clinical or biochemical parameters. The factor of hepatic dysfunction in reducing maintenance doses of FK506 has been emphasized in recent studies.[7]

Statistical analysis. Liver function tests were evaluated using a two-tailed Student's t-test, with unequal variances. Probability values <0.05 were considered statistically significant.

Results

Patient and Graft Survival: In this population of 71 patients, 3 deaths occurred (4.2 percent) while 15 grafts were lost because of the need for retransplantation. Deaths occurred in patients who required FK506 conversion. Two deaths were from sepsis while one death was in a patient with metastatic hepatoma. When the causes of retransplantation were correlated with the indications for entrance into the study, the highest rate of failure was seen in those patients with chronic rejection with or without coexisting hepatitis.

Biochemical response of the liver allograft. The biochemical response of the liver allografts to FK506 was broken down into the specific indications for which the patients were switched to FK506.

A total of 18 patients were analyzed in this group. Four patients were removed from analysis, one because of retransplantation and 3 because of death. This left 14 remaining patients with documented acute rejection. The mean values for total bilirubin, SGOT and SGPT, prior to FK506 were: 8.3 mg/dL, 96 IU/L and 216 IU/L, respectively. These values fell, by the sixth month, to: 0.5 mg/dL, 65 IU/L and 82 IU/L, respectively. Both the alkaline phosphatase (ALK) and gamma glutamyl transpeptidase (GGTP) also fell (215 IU/L to 164 IU/L and 284 IU/L to 123 IU/L, respectively).

Patients with an entrance diagnosis of chronic rejection also had a beneficial response to FK506. Table 2 shows the response of the 33 patients with chronic rejection who were analyzed. Eleven of the 33 grafts failed, requiring retransplantation. This left 22 grafts available for further analysis. The pre-FK506 values for total bilirubin, SGOT and SGPT for the 11 patients who required retransplantation were: 21.0 mg/dL, 282 IU/L and 412 IU/L, respectively. The corresponding values for the patients with chronic rejection who kept their grafts were: 4.3 mg/dL, 204 IU/L and 297 IU/L. While the total bilirubin fell to normal values by six months, (1.1 mg/dL), the average transaminase values were still elevated above normal values at six months (SGOT and SGPT: 97 IU/L and 126 IU/L). The ALK and GGTP levels in the responding group also fell by six months (478 IU/L to 326 IU/L for ALK, and 1316 IU/L to 677 IU/L for GGTP). The liver dysfunction was significantly worse in patients with chronic rejection whose grafts failed as compared to those whose grafts were successfully rescued.

In 20 patients in whom hepatitis was a major process coexistent with chronic rejection, 4 grafts were lost and required retransplantation. The liver function studies were analyzed prior to FK506. Unlike the previous groups, most of the liver function abnormalities were noted as elevations of serum transaminases. No patient was grossly jaundiced. The total bilirubin, SGOT and SGPT prior to and six months after FK506, were: 1.1 mg/dL vs. 0.6 mg/dL, 173 IU/L vs 85 IU/L and 291 IU/L vs 110 IU/L, respectively.

Histologic response of the liver allograft. In each case where histopathologic changes were predominant, the influence of FK506 on the initial findings of rejection or hepatitis were evaluated in serial followup biopsies. The followup period for liver biopsies did not coincide with the followup period for liver function studies. This may account for discrepancies between the magnitude of responses between histologic and biochemical parameters.

In the subset of 18 patients with acute cellular rejection, 14 grafts were still functioning. Ten of the 14 patients had normalization of histologic rejection findings. Four had no changes, although in two patients the followup biopsy was performed within two weeks following FK506 conversion. This is somewhat different than the biochemical parameters, in which all patients improved.

Patients with chronic rejection could be categorized into those patients who failed rescue and required retransplantation and those patients who were successfully rescued. Thirteen of the 22 patients with successful rescues showed histologic improvement while the remaining 9

TABLE 2.
LIVER FUNCTIONS OF PATIENTS WITH CHRONIC REJECTION

Liver Function	Pre-FK506 Values		Six Months Values
	*Failed Rescue**	*Functioning Grafts*	*Functioning Grafts*
TBIL (mg/dL)	21.0	4.3	1.1
SGOT (IU/L)	282	204	97
SGPT (IU/L)	412	297	126
ALK (IU/L)	666	478	326
GGTP (IU/L)	1661	1316	677

*Total number of patients = 33; 11 were failed rescue attempts; 22 were functioning grafts at six months.

showed no changes. The microscopic improvement appeared as lessened portal inflammation and diminished lobular changes or cholestasis. Because of the possibility of sampling errors, it is not possible to state that bile ductule regrowth was seen, however the percentage of duct loss was less in several patients. In the group of patients who failed rescue, the pre-FK506 biopsies generally demonstrated a "late" stage of chronic rejection. Seven of 11 failures were categorized as showing bile duct loss in excess of 50 percent of portal triads. In addition, other microscopic and macroscopic stigmata, including mild portal inflammation, hepatocyte dropout in Zone 3, perivenular fibrosis and obliterative arteriopathy could be found.

Hepatitis occurs as an independent pathologic process. When coexistent hepatitis and rejection occur, it is often difficult to predict which process contributes disproportionately. When both processes exist, treatment is initiated towards the one which may be controlled. Twenty patients with coexistent hepatitis and chronic rejection were switched to FK506. Four grafts were lost and required retransplantation. Hepatitis was a factor in three cases. After FK506 conversion, 10 of the 16 showed stable or improved portal inflammation. Worsening of the histologic hepatitis was seen in six patients characterized by increased lobular disarray, steatosis and hepatocyte necrosis with a paucity of inflammation.

Discussion

This report summarizes our experience on the usefulness of FK506 conversion for patients with refractory rejection while on cyclosporine. The correlation of histologic improvement in patients with biochemical improvement in liver functions confirms that FK506 can arrest and reverse acute rejection and even "early" chronic rejection.[8] Several hallmarks of "late" chronic rejection, including "vanishing bile duct syndrome", obliterative arteriopathy and fibrosis, are high risks factors for failure, however no single histopathologic parameter predicted nonresponse to therapy. Given the high risks associated with retransplantation, we believe that a trial of FK506 conversion therapy may be indicated for refractory rejection, if the patient's condition allows.

An intensive workup is indicated in order to assure that liver function abnormalities are related to rejection and not mechanical or infectious etiologies. We have instituted a rigid protocol, which includes liver biopsies, invasive and non-invasive radiologic examination as well as a careful search for viral infections prior to FK506 conversion. We have found that up to 20 percent of patients will have liver dysfunction unrelated to rejection. Once all causes other than rejection have been ruled out, the timing of FK506 conversion is considered. A trial of FK506 therapy for 2-4 weeks, followed by reevaluation, should be the minimum course. In patients with progressive acute cellular rejection, conversion to FK506 may also require other anti-rejection therapy, dependent on clinical situations. We have employed augmented steroids or OKT3 if an aggressive acute cellular rejection is not stabilized within 24-48 hours after FK506 is started. Liver function tests should normalize or greatly improve within the first two weeks after conversion.

In patients with chronic rejection, it is unrealistic to expect that stigmata of chronic rejection will reverse within a short period of time. In fact, we have had several cases of remarkable recoveries, some of which have taken 2-3 months. In the high risk group of patients with chronic rejection, many of whom had received previous azathioprine, OKT3 and/or high doses of steroids, over 60 percent treated by conversion to FK506 had both clinical and histopathologic responses. This phenomenon, not seen before with patients on cyclosporine, OKT3 or azathioprine, may be related to a hepatotrophic effect of

FK506 on the liver.[9,10] The limitation in the ability to rescue a chronically rejecting liver allograft is likely to be the arterial inflow and the requirement for some residuum of portal biliary structures. Those patients with a diagnosis of chronic rejection who did not respond to FK506 conversion had histopathologic evidence of end stage chronic rejection with obliteration of the vascular lumen and advanced destruction of intrahepatic bile duct structures.

FK506 has been shown to reverse ongoing acute cellular rejection in animal models when started early in the rejection episode.[11] This study demonstrates a marked ability of FK506 to reverse ongoing acute and even "early" chronic rejection. The quality of dose adjustability in the treatment of rejection has always been considered to be limited to steroids. We have recently reported that FK506 can also reverse rejection in other organ systems, including heart,[12,13] kidney[13] and even chronic graft-versus-host disease.[3,13]

FK506 and cyclosporine share many similar biologic properties. Both immunosuppressive agents have specificity towards T cells. Both drugs inhibit interleukin-2 synthesis and, therefore, T cell proliferation.[14,15] The mechanism whereby these drugs inhibit T cell function is thought to be in an inhibition of specific receptors which are peptidyl-prolyl cis-trans isomerases.[16,17] While some of the neurotoxicity, nephrotoxicity and diabetogenic effects of both drugs are similar, there are marked differences. The incidence of hypertension is less while gingival hyperplasia and hirsutism is virtually nonexistent with FK506.

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