15. Early experience with FK 506 in liver transplantation

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Since the introduction of cyclosporine-steroid therapy in 1980 for liver transplantation, 1 year clinical survival rates have approached 70% for most of the common indications for liver replacement and 5 year survival rates are better than 60%. Nevertheless, allograft rejection continues to be a significant cause of retransplantation or death. Clinical rejection occurs in 70% of liver allograft recipients on cyclosporine-steroid therapy. Many patients require treatment with polyclonal or monoclonal antibody preparations to control acute cellular rejection. An increased risk of opportunistic infection, especially from cytomegalovirus, is associated with such therapy.

Nephrotoxicity is a principal and dose limiting side effect of cyclosporine. Chronic renal damage and functional impairment has been shown to occur in many liver transplant patients. Hypertension (secondary to direct renal effects of cyclosporine) requiring antihypertensive therapy is required in over half of these patients. Reduction of cyclosporine dose and/or combination therapy including lower dose cyclosproine, steroids and other agents, usually azathioprine, have been resorted to in order to maintain adequate immunosuppression while reducing intolerable side effects of cyclosporine.

FK 506, a macrolide antibiotic produced from *Streptomyces tsukubaensis*, is produced by the Fujisawa Pharmaceutical Company, Ltd of Japan and is a new immunosuppressive agent which is at least 100 times more potent than cyclosporine.

Animal models

FK 506 was shown to be immunosuppressive *in vitro* by Kino *et al.* (1) and in rats by Inamura *et al.* (2) It has demonstrated a remarkable ability to prolong allograft survival in a number of experimental animal models including renal, liver, and heart allografts (2—11). In studies conducted in Pittsburgh by Todo and co-workers in rats, dogs, and subhuman primates, no evidence of prohibitive toxicity was found. In contrast, studies by Thiru *et al.* reported serious toxicity consisting of widespread arteritis in dogs and baboons (12). However,

studies by Todo *et al.* and Ochiai *et al.* (13) have shown such vascular lesions to be present in non-immunosuppressed dogs after whole organ transplantation or in dogs treated with other agents including cyclosporine. Arteritis has not been seen in baboons treated with FK 506 or in other toxicology studies (10, 11).

Mechanism of action

FK 506 has shown synergism with cyclosporine in experimental systems. The mechanism of action of FK 506, like that of cyclosporine, is not completely understood, but both agents, which are chemically unrelated, show similarities in their effects on T cells.

In studies of the *in vitro* effects of FK 506 on cloned T cell activations, Sawada *et al.* have shown that FK 506 inhibits the response of cloned T cells to concanavalin A and to murine spleen cells in a dose-dependent manner at 40- to 200-fold lower concentrations than cyclosporine. Allo-cytolytic T cell lymphocyte induction from murine thymocytes was inhibited, but not the ability of sensitized T cells to lyse specific target cells. Interleukin-2 (IL2) driven proliferation of activated cloned T cells was not inhibited. However, FK 506 inhibited IL2 secretion and IL2 receptor expression on cloned T cells after stimulation by specific antigen. Cyclosporine has been shown to affect T cell activation in a similar manner but at far higher dosages.

Yoshimura *et al.* (15) have demonstrated FK 506 dose dependent inhibition of IL-2 and gamma-interferon secretion by peripheral human bone marrow cells (PBMC) stimulated with phytohemaglutinin (PHA). FK 506 failed to inhibit B cell stimulating factor 2 (BSF-2) production by PBMC. Both cloned B and T cells, once activated, were not significantly affected by FK 506. Lymphocytes from primary MLR cultured in the presence of FK 506 did not allow for expression of alloantigen activated suppressor cells when used in a dose sufficient to inhibit CTL generation (16). Furthermore, there is evidence that both cyclosporine and FK 506 inhibit transcription of the IL-2 gene and that FK 506, like cyclosporine, acts on an early event in T cell activation (17).

Phase I clinical trials of FK 506 were begun at the University of Pittsburgh with the approval of the Institutional Review Board and the USFDA, in 1989. The initial experience was reported at a special symposium held in Barcelona, Spain, in October 1989 (see *Transplant. Proc.* vol. 22, no. 2, February 1990). The following discussion will summarize the experience reported at that meeting.

Rescue therapy with FK 506

Our experience with the first 40 patients in which FK 506 was used for rescue of patients with intractible rejection or cyclosporine intolerance has been

reported by Fung *et al.* (18) These are among the first human patients to be given FK 506. All patients entered into the study were treated with cyclosporine and prednisone prior to conversion to FK 506. Although some patients were switched to FK 506 purely because of cyclosporine intolerance, most were converted for uncontrolled liver allograft rejection despite maximal therapy with CsA and prednisone and many of these patients also had complications related to cyclosporine including renal dysfunction or hypertension. One patient was withdrawn from FK 506 therapy after 3 days when it was recognized that the patient had recurrent acute hepatitis rather than acute severe graft rejection. The remaining 39 patients were therefore considered treatment failures of conventional immunosuppression.

There were 19 females and 21 males, ranging in age from 5 to 74 years. Liver biopsies were performed in all but 3 patients prior to entry and confirmed the diagnosis of cell mediated rejection. In 6 patients, it was the clinical impression that the biopsy findings underestimated the severity of rejection and in these patients the decision to rescue with FK 506 was based on clinical biochemical parameters. One patient was entered into the trial because of severe steroid intolerance.

Thirty-nine allografts were studied definitively for at least one month or more and all but 8 of these were successfully rescued as judged by histopathologic criteria and liver biochemistry studies. In each case, significant improvement was observed in protocol liver biopsies obtained 2 weeks after the start of treatment with FK 506. Of particular interest was the apparent reversibility under FK 506 of the vanishing bile duct syndrome in those patients with some ducts still remaining in the portal triads. This lesion is usually refractory to treatment with conventional immunosuppression.

Abnormalities in liver function tests also showed significant improvement. An early decline in the cannulicular enzymes (alkaline phosphatase and gamma-GTP) was again of special interest since this correlated with the striking histological improvement seen in patients with vanishing bile duct syndrome. Similar improvements in hepatocellular enzymes (AST and ALT) were also observed in patients with histological improvement seen on protocol biopsies.

One patient subsequently required retransplantation after successful reversal of rejection of his fifth liver graft by FK 506 because of hepatic artery thrombosis resulting from a technical flaw (a local anastomotic stricture). Pathological examination of the resected allograft showed no evidence of residual cell mediated rejection. The patient was given a sixth graft and has since been successfully sustained with this graft under continued immunosuppression with FK 506.

Renal function

Preexisting renal dysfunction was noted in approximately 70% of the patients

entered in this trial. Two patients had prior renal transplants and, in biopsies of both patients, cellular rejection and chronic fibrosis were seen, suggesting that both acute and chronic rejection of the kidney allograft was occurring. Cyclosporine nephrotoxicity was present in most of the other patients treated.

The heterogeneity of the kidneys in patients in this study made it difficult to assess the nephrotoxicity of FK 506. Nine patients had such severe renal dysfunction at the time of switchover to FK 506, including hyperkalemia, that effective cyclosporine therapy was not possible.

Evidence was observed that the effect of FK 506 on renal function is related to an interaction with cyclosporine and that FK 506 augmented cyclosporine nephrotoxicity. With the eventual elimination of cyclosporine after discontinuance of this agent, a reduction in the BUN and Cr was eventually observed in most cases, in spite of therapeutic levels of FK 506. Two of the five patients who required hemodialysis recovered renal function. One patient required institution of hemodialysis, after bilateral renal vein thrombosis was discovered, shortly after institution of FK 506 therapy. Three patients required cadaveric renal transplantation for persistent renal failure during the course of FK 506 therapy.

Primary therapy with FK 506

A phase 1 primary treatment study was begun in the latter half of 1989 and the initial 20 patients were reported by Todo (19). The following is a summary of 33 adult conventional liver transplant recipients who had entered the protocol as of October 15, 1989. In addition, follow-up 3 patients, whose graft rejection could not reversed with the FK 506 'rescue' protocol and who therefore required retransplantation under FK 506, are also included. These are the first human patients to be given FK 506, along with low dose steroids, as their primary immunosuppressive baseline regimen. The results show the remarkably potent immunosuppressive qualities of FK 506 and its relative lack of toxicity in the first several months after transplantation.

A biostatistical survival analysis (Kaplan-Meier) has been performed on the entire series of 33 patients with at lease one month of follow-up and a computer selected group of 81 CsA treated controls matched for age, sex, diagnosis, and clinical urgency (UNOS score). The FK 506 and CsA control patients were well matched for age, sex, and liver disease, but there was a higher proportion of critically ill patients (UNOS score 4) in the FK 506 treated patients than in the CsA treated controls.

A subgroup of the first 20 these 33 patients, for whom follow-up for at least 60 days is available, and a matched group of 20 CsA treated patients were compared for several outcome variables including measures of liver and renal

function, gastrointestinal toxicity, hypertension, the incidence and severity of rejection, and the incidence and severity of major infections.

FK 506, along with low dose steroid therapy, was used for all FK treated patients receiving primary liver allografts, as well as in the three surviving patients initially entered in the 'rescue' protocol and retransplanted under FK 506. FK 506 therapy was initiated following liver transplantation, using a traditional steroid taper over 5 days. (In subsequent phases of the trial the initial high dose steroid taper has been eliminated and the patients simply started on prednisolone at 20 mg per day.) Doses of FK 506 were standardized to 0.15 mg/kg/day as initial intravenous therapy, with conversion to oral FK 506 at ranges of 0.075 mg/kg/day to 0.30 mg/kg/day, based upon blood trough levels and observed side effects. A median of 6 days of intravenous FK 506 was given, before conversion to oral therapy.

Patient survival: Only 2 patients in the initial primary series died. One patient, with primary pulmonary hypertension and unsuspected advanced atherosclerotic heart disease, prior to transplantation, died on postoperative day 15, from heart failure with good liver function and histology. Another patient, who was critically ill (UNOS score 4) at the time of transplantation, developed thrombocytopenia after transplantation and died at 5 days of a hemorrhagic stroke, a well-known complication after liver transplantation, especially in critically ill patients.

Figure 1 presents Kaplan-Meier plots comparing survival of the 33 patients receiving their first liver graft under FK 506 compared to patient survival for the 81 CsA treated and matched historical controls. Thirty-day patient survival for the FK treated patients is 93.9% compared to 87.6% for the CsA treated controls. At a minimum we can say that FK 506 has not had any deleterious

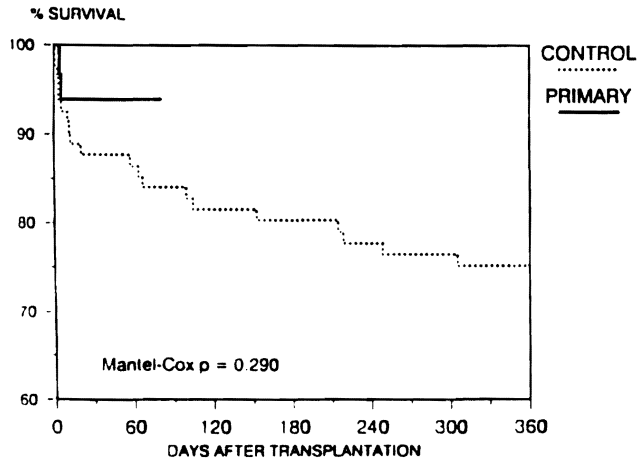

Fig. 1. Patient survival (Kaplan-Meier plot) for primary liver transplantation under FK 506 compared to cyclosporine-steroid treated matched historical controls.

effect on early patient survival. Moreover, although the plots were not yet different at a level of statistical significance, the trend suggested 50% improvement for patients treated with FK 506 compared to those treated with CsA. These early observations have been confirmed and achieved statistical significance in our extended experience recently reported by Todo *et al.* (20).

Liver allograft function

Of the 36 liver allografts analyzed in the protocol (including the 3 retransplantations), all functioned immediately, and no patient required retransplantation for primary graft non-function.

Figure 2 presents Kaplan-Meier plots comparing primary graft survival of the FK 506 treated patients to primary graft survival for 81 grafts in the CsA treated controls. In the FK 506 group, 30 of the 33 grafts are surviving 28 to 80 days after transplantation. Actuarial survival at 30 days is 93.9% For the CsA treated, matched controls, 30 day actuarial survival is 80.0%. The differences in survival had not yet reached statistical significance but the trend suggests a 65% improvement in early graft survival in the FK 506 treated patients. Again, subsequent longer follow-up and a larger case experience recently reported by Todo have confirmed the significance of these observations (20).

Graft function after transplantation was also assessed by serial biochemical measurements. Bilirubin fell to normal levels much more rapidly in FK 506 treated patients than in CsA treated controls (Figure 3). The pattern of hepatocellular enzymes were similar in both groups of patients (Figure 4). Although

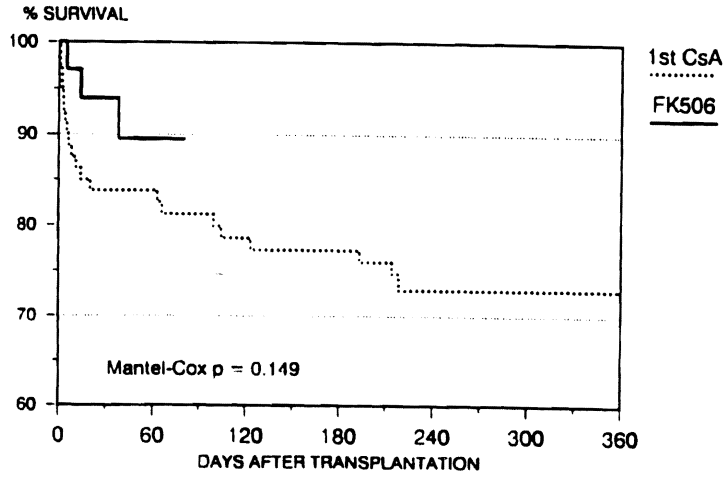

Fig. 2. Graft survival (Kaplan-Meier plot) for primary liver transplantation under FK 506 compared to cyclosporine-steroid treated matched historical controls.

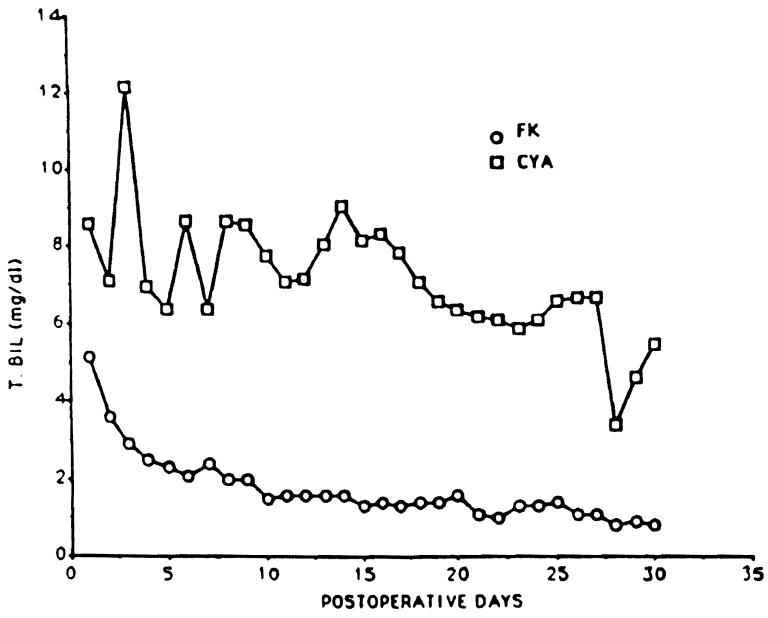

Fig. 3. Postoperative bilirubin for primary recipients of liver grafts treated with FK 506 compared to CsA treated controls (from reference (19)).

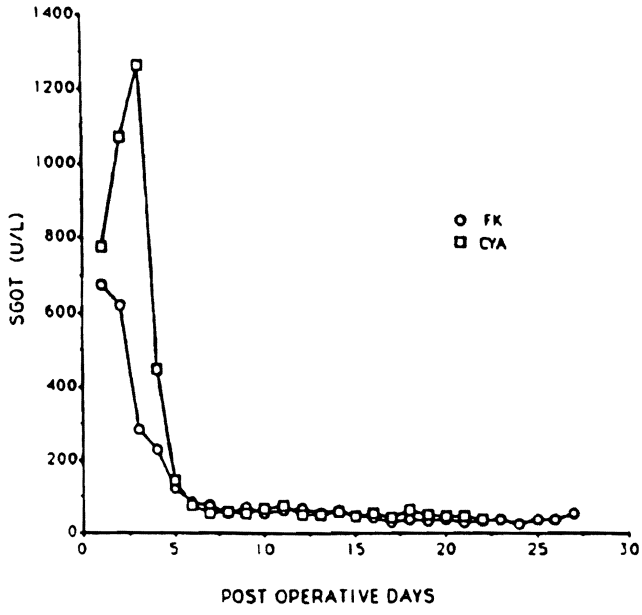

Fig. 4. Postoperative SGOT (AST) for primary recipients of liver grafts treated with FK 506 compared to CsA treated controls (from reference (19)).

initial canalicular enzyme levels were higher in FK 506 treated patients, within 2 weeks after transplantation, these levels fell and remained below those in the CsA treated controls (Figure 5).

Rejection was seen in 60% of CsA treated patients compared to only 10% of the FK 506 treated patients. Additional immunosuppression was required in 18 patients receiving CsA (17 received one or more courses of steroids, while 18 received additional azathioprine, and 11 patients required OKT3).

Two patients experienced significant rejection episodes occurring on post-operative days 12 and 14, respectively. One was successfully controlled with augmented steroids and a 5 day course of OKT3. The other did not respond to steroids or azathioprine, and required retransplantation with a short course of OKT3.

The low frequency and intensity of rejection episodes were demonstrable in protocol liver biopsies. In each case, the absence of lymphocytic cellular infiltrates and preservation of normal portal triad architecture were routinely observed in the first postoperative biopsy performed on the twelfth day after transplantation. In the two patients with clinical rejection, typical findings of cellular rejection were present.

Steroid requirements

The requirement for steroids in patients treated with FK 506 has been less than

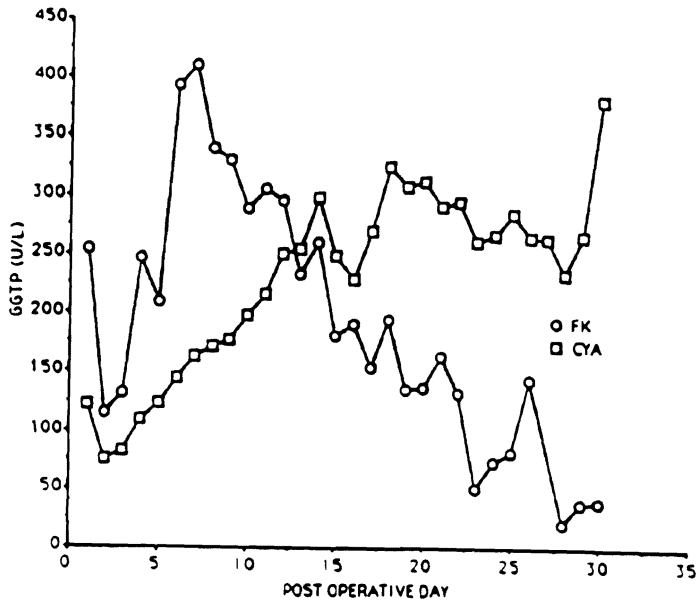

Fig. 5. Postoperative canalicular enzymes (gGTP) for primary recipients of liver grafts treated with FK 506 compared to CsA treated controls (from reference (19)).

that for patients treated with CsA. The mean daily steroid dose in the FK 506 patients was 9 mg/day at one month compared to 19 mg/day for the CsA control group.

Renal function

FK 506 is not a classic nephrotoxin such as the aminoglycosides and does not produce renal cell death and acute renal failure. FK 506 does affect renal function but there are differences from the effects seen with CsA. Elevations in postoperative renal function are not uniform, and promptly decline during the first week following transplantation. We have found a poor correlation (r = 0.45) between FK 506 drug level and serum creatinine (SCr) compared to the strong correlation (r = 0.88) seen between CsA levels and SCr (r = 0.88). Thus, changes in changes in SCr, a dependable indicator of CsA toxicity, is not such a reliable indicator of FK 506 toxicity.

Among the 33 patients treated with FK 506 for their first graft, only one patient, who was on hemodialysis prior to transplantation, required dialysis after transplantation. This pateint, who eventually recovered, was in hepatorenal failure and was on dialysis prior to transplantation. This patient eventually recovered renal function and serum creatinine returned to normal levels while the patient was continued on FK 506. Three patients in the CsA historical control group required hemodialysis during their initial hospital stay.

FK 506 and CsA treated patients were not matched for renal function and serum creatinine (SCr) was higher in the CsA treated controls compared to FK 506 treated patients at all time points, including prior to transplantation. Seventeen FK 506 treated patients and 12 CsA control patients had a SCr less than 1.5 mg/dl prior to transplantation. In this subgroup, pretransplant SCr was less in the CsA treated patients (0.62 $\pm$ 0.24 mg/dl) than in the FK 506 treated patients (0.74 $\pm$ 0.04 mg/dl). However, SCr was greater in the SsA treated subgroup thereafter.

The acute rises in serum creatinine seen in the first patients treated for graft rescue with FK 506 were alarming. However, this is now believed to be a result of the combined severe nephrotoxicity of CsA combined with the milder nephrotoxicity of FK 506. Prior exposure to CsA and its resulting nephrotoxicity leads to a predictable deterioration in renal function, but fortunately this improves after several weeks. FK 506 appears to have much less of a nephrotoxic effect in patients not previously treated with CsA. Thus, patients being converted from CsA to FK 506 are at higher risk of renal dysfunction than patients being treated primarily with FK 506. In patients not suffering from chronic CsA nephrotoxicity, renal function is well preserved.

Hyperkalemia was frequently seen both in CsA and FK 506 treated patients.

Previous studies in CsA treated patients have usually found hyporeninemic hypoaldosteronism and this is also seen in patients treated with FK 506. In addition, there is an association between an increase in SCr and hyperkalemia, suggesting that a reduction in GFR also contributes to the retention of potassium. The hyperkalemia observed was moderate and responded in all cases to fludrocortisone acetate.

Hypertension

Hypertension is the second most troublesome side effect of treatment with CsA. No patient in the primary FK 506 group required addition of antihypertensive medication during the postoperative observation period. One patient with renal artery stenosis had some antihypertensive medications continued, although at a lower level than prior to transplantation.

Gastrointestinal/metabolic effects

The serum cholesterol level was statistically lower in the FK 506 treated group, as compared to the CsA group. Uric acid levels remained in the normal range in patients receiving FK 506. No differences in fasting blood sugar or pancreatic amylase were seen in FK 506 patients compared to CsA treated patients and no significant changes in the appearance of the pancreas have been seen on postoperative CT scans.

Adverse reactions

Unlike the patient population described for CsA to FK 506 conversion, this population of patients receiving FK 506 as primary therapy, had a much lower incidence of side effects. Side effects were assessed by careful interview of the patients by a trained nurse clinician. Although it can be difficult to determine whether subjective patient complaints are attributable to surgery itself or a side effect of medication, there were distinct differences in the incidence of side effects reported by patients taking oral FK 506 when compared to those reported by patients receiving intravenous drug. Eighty-seven percent of patients reported no significant side effects while taking oral FK 506 compared to 42% of patients on intravenous treatment.

The most common side effect of primary intravenous FK 506 administration was headache. The headaches were usually described as mild and required narcotic analgesia in only 2 patients. The headaches responded to symptomatic

treatment and did not require reduction in dosages. Switching to oral administration relieved the headache in all but 1 patient.

The next most frequent side effects of intravenous therapy were gastroenterologic, especially nausea. Treatment consisted of antiemetics, and spontaneous resolution of these symptons occurred in all patients following conversion to oral therapy. With intravenous therapy, mild anorexia was sometimes associated with nausea. Oral intake was adequate in all patients.

No adverse hemodynamic reactions, such as hypotension or other alterations in cardiac performance, were noted during the oral or intravenous administration of FK 506. Detailed cardiovascular profiles were developed during the initial intravenous infusion with FK 506. No patient required augmented antihypertensive medications as an outpatient.

Other side effects seen with intravenous FK 506 were a feeling of warmth and flushing. Less frequent side effects were rash, chest pain (without EKG changes), anxiety, abdominal cramping, night sweats, fatigue, photophobia, and blurred vision. These symptom complexes were also markedly reduced following conversion from intravenous to oral FK 506.

Infection

The incidence of serious infections (defined as life-threatening infections), was not increased with the use of FK 506 when compared to CsA. In fact, the incidence of bacterial infections was low, occurring in only 4 patients in each group. Transient bacteremia from an indwelling intravenous catheter was seen in 1 patient on FK 506 and resolved with removal of the catheter. Two wound infections were seen, one mild and the other extending into subfascial planes which required drainage. One patient, treated for staphylococcal endocarditis prior to transplantation, developed spontaneous staphylococcal peritonitis while on FK 506. Viral infections, in particular cytomegalovirus (CMV) infections, were seen in 2 patients in the initial 20 FK 506 treated primary patients, as compared to 5 patients on CsA. These CMV infections were clinically mild and responded to treatment with gancyclovir.

Charges and hospital stay

An additional measure of the relative effectiveness and safety of FK 506 has been its impact on the length of hospital stay and related hospital charges after liver transplantation when compared to similar measures for CsA treated patients. The findings are even more striking when it is remembered that there was a significantly higher proportion of critically ill (UNOS class 3 and 4)

patients in the FK 506 treated group than in the CsA treated control group. Patients treated with CsA tended to stay in the hospital twice as long and accrued total bed charges almost three times greater than FK 506 patients (21).

Pharmacologic monitoring

Because of the possibility that FK 506 and cyclosporine would be used together, pharmacokinetic studies were performed with each drug individually, and then in combination, with both the intravenous and oral administration (22). Pharmacolinetic data of FK 506 suggests that the metabolism is primarily hepatic in nature. Peak levels in the 5—10 ng/ml range could be detected 1—2 hours after intravenous administration. The terminal blood half-life appears to be 12 hours after a redistribution phase following an intravenous dose. The nature of absorption, peak levels and possibily the terminal half-life seem somewhat different following an oral dose. A relatively flat peak is generally seen, and the absorption of the oral dose is estimated at 30%.

Both polyclonal and monoclonal immunoassay techniques have been perfected to monitor both blood and tissue levels of this drug and its metabolites.

Conclusion

FK 506 is an extraordinary immunosuppressive agent that has shown great promise in the early clinical trials in liver transplantation. It is much more potent than cyclosporine and, although it has a similar spectrum of side effects, it nevertheless appears to be much better tolerated than CsA. In particular, nephrotoxicity appears to be much milder with FK 506. Equally impressive is the ability of FK 506 to arrest the vanishing bile duct syndrome of chronic liver rejection at a later stage than with any previously available agent. Finally, the drug is much more effective than CsA in preventing acute rejection early after transplantation which permits early reduction in steroid dosage, avoids the need for use of antibody therapy and the penalties associated with the use such agents, and permits earlier discharge from the hospital after transplantation.

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References

1. Kino, T., Hatanaka, H., Inamura, N. *et al.*: FK 506, a novel immunosuppressant isolated from Streptomyces. II. Immunosuppressive effect of FK 506 *in vitro*. *Transplant. Proc.* **19** (Suppl. 6), 64—67 (1987).
2. Inamural, N., Nakahara, K., Kino T. *et al.*: Prolongation of skin allograft survival in rats by a novel immunosuppressive agent, FK 506. *Transplantation* **45**, 206—209 (1988).
3. Gudas, V.M., Carmichael, P.G. and Morries, R.E.: Comparison of the immunosuppressive and toxic effects of FK 506 and cyclosporine in xenograft recipients. *Transplant. Proc.* **21** (1 Pt 1), 1072—1073 (1989).
4. Ochiai, T., Nakajima, K., Nagata, M. *et al.*: Effect of a new immunosuppressive agent, FK 506 on heterotopic allotransplantation in the rat. *Transplant. Proc.* **19**, 1284—1286 (1987).
5. Ochiai, T., Nagata, M., Suzuki, T. *et al.*: Studies of the effects of FK 506 on renal allografting in the beagle dog. *Transplantation* **44**, 729—733 (1987).
6. Ochiai, T., Nakajima, K., Nagata, M. *et al.*: Studies of the induction and maintenance of long term graft acceptance by treatment with FK 506 in heterotopic cardiac allotransplantation in rats. *Transplantation* **44**, 734—738 (1987).
7. Lim, S.L.M., Thiru, S. and White, D.J.G.: Heterotopic heart transplantation in the rat receiving FK 506. *Transplant. Proc.* **19** (Suppl. 6), 68 (1987).
8. Todo, S., Demetris, A.J., Ueda, Y. *et al.*: Canine kidney transplantation with FK 506 alone or in combination with cyclosporine and steroids. *Transplant. Proc.* **19** (Suppl. 6), 57—62 (1987).
9. Todo, S., Podesta, L., Chapchap, P. *et al.*: Orthotopic liver transplantation in dogs receiving FK 506. *Transplant. Proc.* **19** (Suppl. 6), 64—67 (1987).
10. Todo, S., Ueda, Y., Demetris, A.J. *et al.*: Immunosuppression of canine, monkey, and baboon allografts by FK 506 with special reference to synergism with other drugs, and to tolerance induction. *Surgery* **104**, 239—249 (1988).
11. Todo, S., Demitrsi, A., Ueda, Y. *et al.*: Renal transplantation in baboons under FK 506. *Surgery* **106**, 444—451 (1989).
12. Thiru, S., Collier, D. St J. and Calne, R.: Pathologic studies in canine and baboon renal allograft recipients immunosuppressed with FK 506. *Transplant. Proc.* **19** (Suppl. 6), 98—99 (1987).
13. Ochiai, T., Sakamoto, K., Gunji, Y. *et al.*: Effects of combination treatment with FK 506 and cyclosporine on survival time and vascular changes in renal-allograft-recipient dogs. *Transplantation* **48**, 193—197 (1989).
14. Sawada, S., Suzuki, G., Kawase, Y. and Takaku, F.: Novel immunosuppressive agent, FK 506. In vitro effects on cloned T cell activation. *J. Immunol.* **139**, 1797—1803 (1987).
15. Yoshimura, N., Matsui, S., Hamashima, T. and Oka, T.: Effect of a new immunosuppressive agent, FK 506, on human lymphocyte responses in vitro. II. Inhibition of the production of IL2 and gamma-IFN, but not B cell-stimulating factor 2. *Transplantation* **47**, 351—359 (1989).
16. Yoshimura, N., Matsui, S., Hamashima, T. and Oka, T.: Effect of a new immunosuppressive agent, FK 506, on human lymphocyte responses in vitro. II. Inhibition of expression of alloantigen-activated suppressor cells, as well as induction of alloreactivity. *Transplantation* **47**, 351—356 (1989).
17. Tocci, M.J., Matkovich, D.A., Collier, K.A. *et al.*: The immunosuppressant FK 506 selectively inhibits expression of early T cell activation genes. *J. Immunol.* **143**, 718—726 (1989).
18. Fung, J.J., Todo, S., Jain, A. *et al.*: Conversion from cyclosporine to FK 506 in liver allograft recipients with cyclosporine-related complications. *Transplant. Proc.* **22** (Suppl. 1) 6—12 (1990).
19. Todo, S., Fung, J.J., Demtris, A.J., *et al.*: Early trials with FK 506 as primary treatment in liver transplantation. *Transplant. Proc.* **22** (1 Suppl. 1), 13—16 (1990).

20. Todo, S., Fung, J.J., Starzl, T.E. *et al.*: Liver, kidney, and thoracic organ transplantation under FK 506. *Ann. Surg.* (in press).
21. Staschak, S., Wagner, S., Block, G. *et al.*: A cost comparison of liver transplantation with FK 06 or CyA as the primary immunosuppressive agent. *Transplant. Proc.* **22**, 47—49 (1990).
22. Venkataramanan, Jain, A., Cadoff, E. *et al.*: Pharmacokinestics of FK 506: Preclinical and clinical studies. *Transplant. Proc.* **22**, 52—56 (1990).