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EDITORIALS & REVIEWS

FK 506: a new immunosuppressive agent for organ transplantation. Pharmacology, mechanism of action and clinical applications

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Control of the immune response directed at the allograft is the ultimate goal of the immunosuppression utilized to achieve a successful organ transplant. The initial approach to immunosuppression was the use of high doses of glucocorticoids, often in combination with cytotoxic agents such as azathioprine and/or cyclophosphamide (1-3). In 1976, following the discovery of the T cell specific immunosuppressant Cyclosporine A (CsA) (4), the immunosuppressive therapy utilized at transplant centers entered a new era. A large number of both basic and clinical studies demonstrated a dramatic improvement in the outcome of whole organ transplantation, both in terms of allograft survival and patient quality of life with such agents. Despite this major step forward, the failure to completely control the rejection process in some patients and the presence of side effects, such as nephrotoxicity (5), hypertension (6-8), hepatotoxicity (6-8), diabetogenicity (6-8), central nervous system dysfunction (6-8), have remained as troublesome problems associated with the use of immunosuppressive therapy required for continued organ engraftment. Since the introduction of CsA, many studies have been performed in an effort to identify new and more powerful immunosuppressive agents which would further improve allograft acceptance (tolerance) without inducing the toxic side effects of existing immunosuppressive agents.

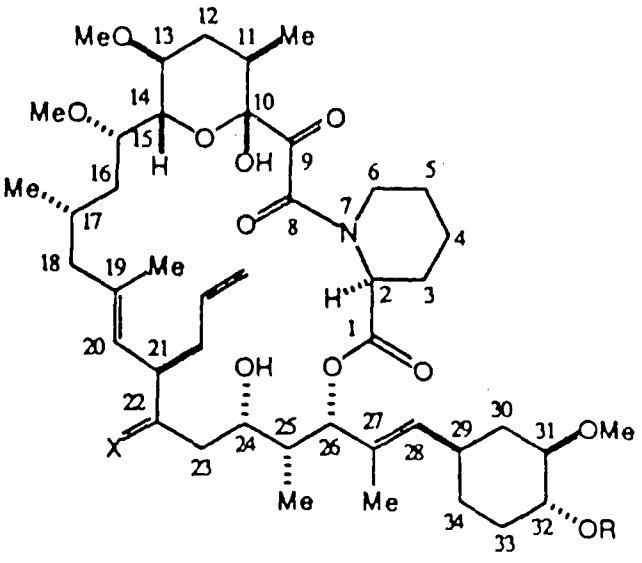
One of these new agents, FK 506, an hydrophobic antibiotic of the macrolide family (Fig. 1), was isolated in 1985, in Japan, by the Fujisawa Pharmaceutical Company from a fermentation broth of a soil organism, *Streptomyces Tsukubaensis* (9,10). Initial in vitro and in vivo studies demonstrated that FK 506 was effective in suppressing the immune response having an ED_{50} of approximately 0.1 nM, which is close to 100 times more active than Cyclosporine A in similar assays (10-14).

Subsequent studies have demonstrated that FK 506 successfully inhibits hepatic (15,16), renal (17), small intestine (18) and cardiac (15,19) rejection in animal models of organ transplantation (20). Since these early studies were reported many additional biochemical and immunological studies have been performed to assess the clinical usefulness of this new drug (21). Beside the immunological activity, FK 506 and CsA have shown powerful hepatotrophic properties both in "in vivo" and "in vitro" experiments (22,23).

BIOCHEMICAL SITE OF ACTION

FK 506, like CsA and Rapamycin (another new immunosuppressive agent that also inhibits T cell activation at con-

FK 506



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centrations comparable to those of FK 506), binds with high affinity to cytoplasmic receptors termed immunophillins (immunosuppressant binding proteins). The predominant cytosolic FK 506 binding protein is termed FKBP. It has been isolated and structurally characterized by Schreiber et al (24). Like cyclophillin, the major cytosolic binding protein for CsA, FKBP has rotamase activity (folds peptide substrates). Despite their quite similar common enzymatic properties, these two immunophillins have very different primary structures. The geometry of the immunophillin-drug complex has been invoked as a biological effector that inhibits T cell activation (24).

IMMUNOMODULATING PROPERTIES OF FK 506

FK 506 inhibits T cells activation by mechanisms similar to those identified for Cyclosporine A. Although structurally unrelated, CsA and FK 506 share several putative mechanisms of action at both the cellular and the molecular level. T lymphocyte activation occurs following a recognition event that is initiated via the T-cell receptor/CD-3 complex combined with one or more additional signals provided by accessory cells and/or cytokines. In this process, a complex series of events takes place; these include an increase in intracellular free calcium, phosphorylation of cytosolic proteins, activation of protein kinase C and increased phosphoinositide turnover (25). Neither FK 506 or CsA appear to affect these early events following T cell activation (26). Once initiated, these biochemical processes lead to the coordinate expression of a set of gene products critical for lymphocyte growth and differentiation.

Current evidence suggests that both CsA and FK 506 inhibit the induction of a discrete set of lymphokine genes which include IL-2, IL-3, IL-4, GM-CSF, TNFa and interferon γ (27) at the transcriptional level. Both FK 506 and CsA have been shown to directly inhibit transcription of the IL-2R gene (28). Taken together, these data suggest that FK 506 and CsA inhibit T cell receptor-mediated signal transduction pathways that are distal to the early membrane-associated events described above but that are proximal to regulatory nuclear transcriptional factors (29).

HEPATOTROPHIC PROPERTIES OF FK 506

Kim (30) and Makowka (31) have reported that CsA enhances the regenerative response induced by partial hepatectomy (PH). Subsequently, FK 506 has been shown to stimulate hepatic regeneration in rats following a PH (23). The growth enhancement achieved with FK 506 is greater than that obtained with CsA. Importantly, the growth stimulatory activity obtained with both CsA and FK 506 is organ-specific in that it does not alter the proliferative response of the kidney following a unilateral nephrectomy or that of the remnant intestine following a 40% intestinal resection (22). Experiments performed in nude rats have ruled out a direct effect of immune modulation via T cells in the control of the regenerative process in response to either CsA and FK 506 (24, 32-34). FK 506 and CsA bind to intracellular proteins, immunophillins, to form complexes which modulate a wide variety of calcium dependent signal transduction pathways that affect T-lymphocytes (35-37). The interaction of these two agents with immunophillins also modulates the process of liver cell proliferation but not through an alteration in intracellular calcium levels. In contrast, Rapamycin, another macrolide antibiotic that binds to the same immunophillin as does FK 506, is anti-hepatotrophic and inhibits hepatic regeneration in rats subjected to a partial hepatectomy (38,39). These observations constitute the first physiologic evidence that the immunophillin network regulates growth control (33).

PHARMACOKINETIC AND PHARMACODYNAMIC PROPERTIES

Experimental Studies In most of the available studies concerning the pharmacokinesis of FK 506, an enzyme-linked immunosorbant assay (ELISA) has been used for the quantitation of the drug (40). Tamura et al (40) showed that the FK 506 serum levels in dogs range between 0.1 and 0.4 ng/ml following a dose of 0.1 mg/Kg per os. After administration, FK 506 is distributed in various organs including the lungs, spleen, heart and kidneys (41). The majority of the drug is metabolized by the liver and is excreted in bile, urine and feces within 48 hrs of administration (41). FK 506 has been shown to down-regulate the activity of certain Cytochrome P-450 systems (41). In addition FK 506, like CsA, possesses both hepatotrophic and hepatoprotective properties (22,42). The coadministration of CsA and FK 506 in vitro reduces the effective level of each required to produce immunosuppression and suggests a synergy might exist between the two drugs (43). Clinically however, enhanced renal toxicity, rather than immunosuppressive synergy has been observed when both agents are used simultaneously.

Clinical Studies The toxic effects of FK 506 have limited the available studies addressing the important issue of dose and route of administration of the drug. In the first clinical trial of FK 506 for liver transplantation (44) the following protocol was utilized: 0.15 mg/Kg was administered intravenously within one hour after the liver graft was revascularized. This was followed by an infusion of FK 506 at the dose of 0.075 mg/Kg/12hrs until the patient could eat at which time the patient was switched to oral drug at a dose of 0.15 mg/Kg/12 hrs. Peak plasma levels are observed immediately after the intravenous administration and decline slowly for 24 hrs (41). Plasma through levels range about 1 ng/ml (41). Following oral administration of the drug, absorption is quite variable: peak plasma levels of 0.4-3.7 ng/ml occur after an oral dose of 0.15 mg/Kg (41). Similar to what occurs with

CsA, impaired liver function alters the absorption and metabolism of FK 506 (45,46). Patients with hepatic dysfunction experience higher FK 506-levels, have an increased FK 506 half life and reduced clearance than patients with good liver function (46). As a result, liver transplant recipients, whose grafts do not function well and/or fail, can experience very high plasma levels with associated neurotoxicity and renal failure following i.v. administration of the drug. Even with good hepatic function, it is necessary to carefully control the plasma levels of the drug, maintaining the level below 3 ng/ml to avoid toxicity. Failure to observe these rules constitutes a serious risk for patient morbidity. Currently at the University of Pittsburgh smaller doses of FK 506 as compared to those used in the initial clinical trials are being used (0.075 or 0.10 mg/Kg/day; i.v. or p.o. respectively).

CLINICAL APPLICATIONS

Organ Transplantation Preliminary clinical studies have reported that FK 506 is highly effective in increasing survival in both solid organ and skin transplantation (47). These observations confirm the published reports of enhanced graft survival in animal models of transplantation (15-20). Importantly and quite different from that achieved with CsA, FK 506 is capable of reversing acute as well as chronic rejection in liver, kidney and heart transplantation (15-20, 48). Equally important is the fact that FK 506 based immunosuppression requires less steroids than does conventional immunotherapy with CsA (47).

Clinically, FK 506 was introduced in February 1989 at the University of Pittsburgh. Initially it was used to treat liver recipients who were facing intractable rejection or excessive drug toxicity under conventional CsA-based immunosuppression. This "rescue" therapy with FK 506 was shown to be effective in reversing rejection in 7 out of 10 patients, who were unresponsive to the highest permissible immunotherapy under CsA combined with steroids and OKT3 (47). Following this experience, FK506 was used as a primary immunosuppressive agent, initially for liver transplantation and subsequently for renal, heart, lung, small bowel and pancreatic islet cell transplantation (49-55).

Liver transplantation Recipient survival rates were improved significantly over that of 325 historical controls in the first 125 patients receiving FK 506 for both primary hepatic transplantation and retransplantation (91.2% vs 78.2% and 88.8% vs 75.4% at 6 months and one year, respectively) (both $p<0.01$). For graft survival, the rates at 6 months and 1 year were 84.8% vs 72.6% and 80.8% vs 68.5% respectively (both $p<0.01$) (49).

In order to assess the true rates of patient and graft survival and rejection as well as to define the incidence of adverse side effects of FK 506, a prospective randomized trial was [illegible] in a head to head competition using only ideal transplant recipients. This study included 111 patients, 57 on FK 506 and 54 on CsA and demonstrated a 1 year survival rate of 95% for the FK 506 group vs 85% for the CsA treated group. Corresponding graft survival rates were 93% and 77%, respectively. After a median follow-up of 256 days (range 41-405), 44% of the patients in the FK 506 group were still free of a rejection episode, compared to only 23% of the CsA treated group ($p<0.01$). The use of OKT3 as adjuvant immunosuppressive agent was required in 43% of the CsA patients but only 22% of the FK 506 treated patients ($p<0.01$). Moreover, FK 506-treated patients required a statistically significant lower amount of steroid and, as a result, had a lower incidence of untoward side effects such as hypertension, hyperkalemia and hirsutism than did the group treated with conventional CsA-based immunosuppressive therapy. The incidence of hypertension was statistically higher in the CsA treated patients (33%) as compared to the FK 506 (27%) ($p<0.05$). No significant difference between the two groups was observed for renal dysfunction, infectious complications and frequency of new onset carbohydrate intolerance (49, 53).

Kidney transplantation The first kidney transplant using FK 506 was performed at the University of Pittsburgh in March 1989. Since then 411 kidneys have been transplanted in Pittsburgh through January 1991. Of these, 202 received FK 506 as their primary immunosuppressive therapy. No substantial difference between FK 506 and CsA treated patients for the one year actuarial patient and graft survivals (91% vs 94% and 75% vs 81% respectively) was observed. The incidence of graft rejection episodes (57% vs 54%) and of CMV infection (14.6% vs 13.8%) were similar between the two groups; twenty one percent and 38% of the recipients respectively required OKT3.

The principal difference between the two groups was in the rate of use of prednisone, 38% of the FK 506 recipients were not requiring prednisone as compared to none of the CsA group. The use of anti-hypertensive medications was similarly reduced in the FK 506 group with 52% requiring no anti-hypertensive drugs as compared to 29% of-the CsA group.

Another advantage for the FK 506 treated group was the reduced mean serum cholesterol level (193±44 mg/dl) in the FK 506 group as compared to the CsA group (231±64 mg/dl) (3, 55).

Heart transplantation FK 506 as the primary immunosuppressive therapy has been used in 42 adult and 13 children receiving heart transplants at the University of Pittsburgh since October 1989. Overall patient survival has been 93% after a mean follow-up of 400 days. Actuarial freedom from rejection has been 65%. Compared to historical controls treated with CsA, patients treated with FK 506 experience a [illegible]

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diminished incidence of hypertension (15% vs 70%) (all $p<0.001$) (54).

Small bowel transplantation Since the first small bowel transplantation was performed by Lillehei in the early 1960's, it has been evident from a purely technical point of view that intestinal transplantation is a practical possibility. In contrast with the kidney and liver transplantation, however, no immunosuppressive agent has been shown to be truly effective in preventing rejection within days of the transplant procedure. Additionally, intestinal transplantation presents a very high risk for graft-versus-host disease (GVHD). Recently FK 506 has been shown to be an effective immunosuppressive agent in a rat model of intestinal transplantation (18). These experimental studies have been confirmed in human intestinal transplantation (56).
The preliminary results at the University of Pittsburgh are encouraging and have shown FK 506 to be a useful agent in this type of transplant. Moreover, no GVHD has been observed following intestinal transplantation with FK 506 (56).

Immune-related disease and FK 506 The rationale for the use of FK 506 in the clinical management of autoimmune diseases is the observation that: 1) many of these diseases are T-cell mediated and FK 506 is a powerful T-cell inhibitor and 2) most if not all T-cell mediated putative autoimmune disorders have been shown to be responsive to CsA therapy, but concern about CsA toxicity, have limited its use. Preliminary results with FK 506 have shown a beneficial effect in 10 patients treated for severe, recalcitrant *chronic plaque forming psoriasis* (two of whom also had psoriatic arthritis) (57); in 3 patients with severe *pyoderma gangrenosum* associated with inflammatory bowel disease (IBD) (58) and in 7 patients with the nephrotic syndrome caused by *steroid-resistent focal sclerosing glomerulonephritis* where FK 506 markedly reduced the proteinuria without altering overall renal function (59).
As a result of the success of these preliminary studies, several randomized clinical trials (new onset type I diabetes, scleroderma, autoimmune glomerulonephritis, systemic lupus erythematous, polymyositis-dermatomyosius, reumatoid arthritis, psoriasis, pyoderma gangrenosum, Crohn colitis, ulcerative colitis, primary biliary cirrhosis, primary sclerosing cholangitis, autoimmune chronic acute hepatitis and uveitis) are presently underway at the University of Pittsburgh with this agent (60).

UNTOWARD EFFECTS OF FK 506

As with all conventional immunosuppressive therapies, the major untoward effects of FK 506 can be segregated into 4 principal groups: renal dysfunction, diabetogenic effects, neurotoxicity and infections.

Renal dysfunction Although FK 506 is not a typical nephrotoxin, it does reduce glomerular filtration, probably by altering renal cortical hemodinamics. It also has some adverse tubular effects. Hyperkalemia is a frequent complication of FK 506 therapy and is due either to an effect on mineralcorticoid secretion either at the level of the adrenal or juxtaglomerular apparatus or an alteration in mineralcorticoid activity at the level of the renal tubules. With high levels of FK 506, renal failure can occur necessitating hemodialysis. The reported incidence of hemodialysis in liver transplant recipients is 4% and appear to be a unique problem for these patients, as it is not seen in either heart or heart/lung recipients (53, 61).

Glucose metabolism New onset diabetes mellitus requiring insulin occurs at a rate of about 18% with the use of FK 506. This rate is similar to that experienced with CsA.
Glucose intolerance in response to FK 506 administration appears to be due to changes in the peripheral sensitivity to insulin as well as a reduced insulin secretion rate by B cell in pancreatic islets in response to a hyperglycemic stimulus (53, 62, 63).

CNS dysfunction CNS dysfunction following FK 506 treatment is rare. When it occurs, however, it can be severe (53, 64). A reversible expressive aphasia, new onset seizures and paranoid psychosis have all been reported in liver recipients taking FK 506. This unique susceptibility of liver recipients to neurotoxicity following FK 506 administration may depend in part to an underlying metabolic dysfunction that is due to the presence of preexisting hepatic encephalopathy and/or a disruption of the blood brain barrier associated with hepatic encephalopathy that persists during the perioperative period. Minor neurotoxic complications of FK 506 include: insomnia, tremors, headache, hyperesthesia, blurred vision, tinnitus, dizziness and nightmares (53, 64).

Infections The incidence of bacterial infections during FK 506 immunosuppression is low (65). Viral infections, such as cytomegalovirus, occur in 20% of transplant patients (53, 65, 66). Fortunately, the CMV infections seen in transplant recipients using FK 506 are typically mild and respond rapidly to gancyclovir administration. To date no CMV infection has been reported in a patients treated with FK 506 for a non-transplant indication (53, 65, 66).
Despite its increased immunosuppressive activity, FK 506 has not been shown to have an increased risk of lymphoproliferative disorders (LPD) associated with its chronic use as a primary immunosuppressive agent. Moreover, when compared to CsA, the rate of LPD with FK 506 is 1/3 less than that seen in organ graft recipients treated with CsA (53, 67). Hypertension is not a serious problem with FK 506 therapy, a fact that distinguishes it from CsA (53). Gingival hyperplasia and excessive hair growth are also not seen with FK 506 administration (68). Importantly, hyperuricacidemia and hypercholesterolemia are not seen with FK 506 ad-

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