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In Vitro Assessment of FK 506 Immunosuppressive Activity in Transplant Patients

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FK 506 has established itself as a promising immuno-suppressive drug in organ transplantation and in the treatment of autoimmune disease. Therapeutic monitoring of plasma concentrations of FK 506 is essential to ensure appropriate dosage for adequate immunosuppression and to minimize potential side effects. Routine monitoring of FK 506 plasma concentration is performed with an enzyme-linked immunoassay (ELISA) previously developed by Tamura et al[1] and modified by Cadoff et al.[2] Plasma FK 506 levels also have been measured with an in vitro bioassay.[3] This assay is based on the inhibition of the alloantigen driven proliferation of cloned alloreactive T cells. These activated lymphocytes show a narrow sensitivity range to FK 506 and the IC50 is 0.07 to 0.12 nmol/L. In liver allograft recipients, the FK 506 levels as determined by bioassay are consistently lower than those measured by ELISA (Fig 1). These results suggest that the plasma may contain biologically less active FK 506 metabolites, which can be detected by ELISA.

FK 506 METABOLISM

The liver appears to be the primary site of FK 506 metabolism.[4] Venkataramanan et al[5] have isolated several FK 506 metabolites from human and rat bile samples. These in vivo generated metabolites represent largely the products of demethylation and hydroxylation of FK 506. A total of four FK 506 metabolite fractions have been isolated by high performance liquid chromatography (HPLC) of rat bile and three fractions were tested for their immu-
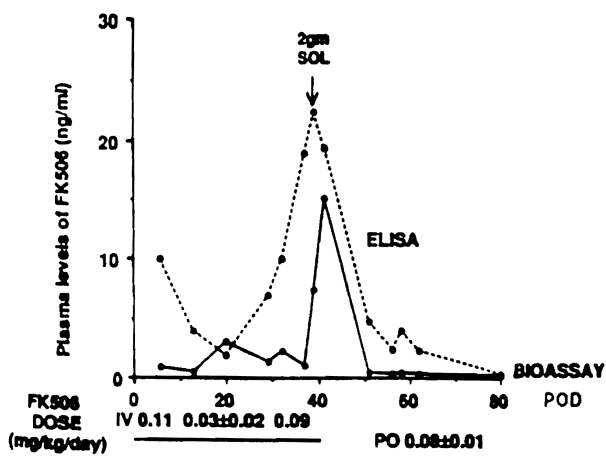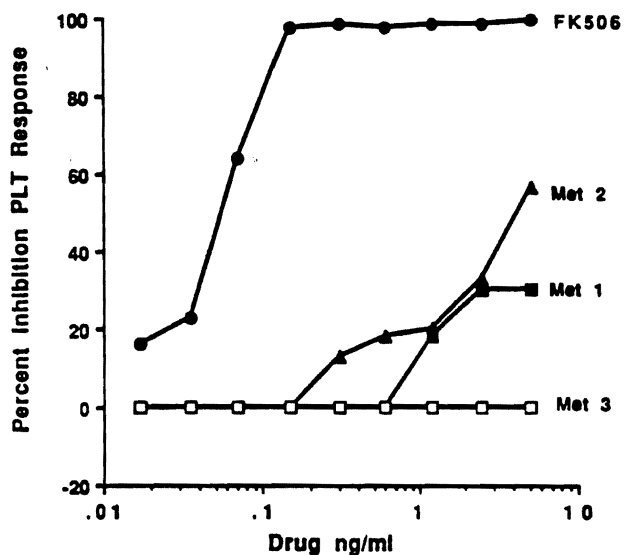

Fig 2. Effect of different concentrations of FK 506 and metabolites Met 1, Met 2, and Met 3 on donor-specific PLT activity of alloreactive T-cell clone DB29. The PLT response of DB29 was 53,324 ± 3,219.

nosuppressive activity in an in vitro bioassay with cloned alloreactive T cells. Only one of the three fractions showed significant inhibition of the PLT activity of an alloreactive clone, but at a 100-fold higher concentration than that of the parent drug (Fig 2). Christians et al[6] identified nine metabolites of FK 506 when FK 506 was incubated with human liver microsomes. Only two out of nine metabolites exhibited significant immunosuppressive activity in vitro, but the IC50 values were only 10% or less of the IC50 of FK 506. These investigators have developed a specific HPLC/ultraviolet (UV) assay for quantification of FK 506 and its metabolites in blood, bile, urine, and microsomal

Fig 1. FK 506 levels in plasma measured by the bioassay and ELISA in a liver transplant recipient.

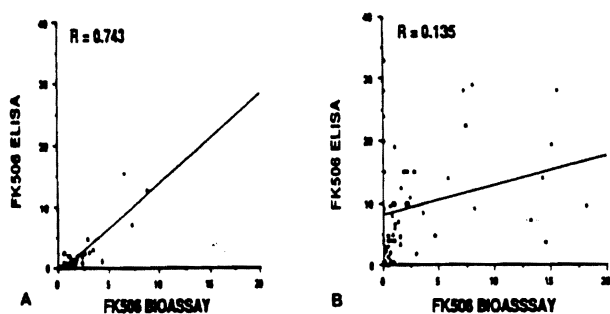© 1991 by Appleton & Lange
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Fig 3. Correlation between ELISA and bioassay. FK 506 plasma levels (ng/mL) in **(A)** stable liver transplant recipients (classes I + II) and **(B)** liver transplant recipients with prolonged hepatic dysfunction (classes III + IV).

preparations.[7] The in vivo and in vitro models provide opportunities to enhance our understanding of the metabolic pathways of FK 506 and what conditions exert major influences on FK 506 metabolism and ultimately, the clinical efficacy of FK 506.

CONDITIONS THAT AFFECT FK 506 METABOLISM

Close pharmacologic monitoring of FK 506 is essential to the optimal management of the immunosuppressed patient. Abu-Elmagd et al[8] have recently demonstrated that the liver represents a dominant influence on FK 506 pharmacokinetics. In liver transplant patients, prolonged hepatic dysfunction postsurgery causes a rapid and persistent rise in plasma levels of FK 506 as measured by ELISA even after dose reduction. FK 506 plasma levels correlate directly with serum bilirubin levels, suggesting that liver dysfunction interferes with FK 506 metabolism.[8] As illustrated in Fig 1, in liver transplant patients the FK 506 levels as measured by the bioassay are consistently lower than FK 506 levels measured by ELISA. A comparative analysis with five patients categorized as class I[8] has shown a good correlation (r = .74) between both levels and the bioassay values are about two-thirds of the ELISA values (Fig 3, left panel). In contrast, in a group of patients with hepatic dysfunction (classes III and IV, see ref 8) we were unable to demonstrate a significant correlation (r = .13) between plasma FK 506 levels measured by both assays (Fig 3, right panel). Most plasma with high ELISA values showed much lower bioassay values. These observations suggest that biologically inactive FK 506 metabolites may accumulate in the plasma in liver transplant patients with severe hepatic dysfunction. Studies are in progress to further analyze the FK 506 metabolism patterns by HPLC analysis.[9]

Coadministration of other drugs may also affect FK 506 metabolism by enhancing or inhibiting the cytochrome P-450 system. Administration of steroids to patients on FK 506 increases FK 506 ELISA levels without a proportional increase in FK 506 bioassay concentrations.[3] This may be related to the inhibitory effect of steroids on the biliary excretion of FK 506 metabolites.[4] Similar observations

have been made with cyclosporine (CyA)-treated patients in whom a steroid bolus caused an increase in blood CyA levels measured by radioimmunoassay, and CyA levels determined by HPLC were unaffected.[10]

Consideration must also be given to other drugs that are known to inhibit hepatic drug metabolism. A prime example is the administration of erythromycin, which is associated with significant increases in FK 506 levels measured by ELISA and bioassay. Even after the doses of FK 506 had been lowered, the trough plasma levels of FK 506 remained elevated. We have also observed that ketoconazole administration may also be associated with increased FK 506 plasma levels.[5] Further studies are necessary to evaluate the effects of various drugs on FK 506 metabolism. A better understanding of drug interactions will improve the management of allograft recipients on FK 506 allowing prompt dose adjustments when required. Clinical experience has shown that therapeutic FK 506 levels of 0.8 to 2 ng/mL are required to control allograft rejection.[5] Although rejection is indicated by clinical parameters and laboratory measurements of allograft function, the biopsy histology generally provided a definitive diagnosis. Allograft biopsies with histologic rejection show infiltration by lymphocytes that can now be readily propagated with relatively simple culture techniques.[11]

PROPAGATION OF LYMPHOCYTES FROM TRANSPLANT BIOPSIES- ASSOCIATION WITH FK 506 PLASMA LEVELS

The in vitro culturing of lymphocytes from transplant biopsies is based on the concept that the allograft is infiltrated by activated T cells that proliferate in the presence of interleukin-2 (IL-2). In heart transplant patients on CyA immunosuppression, lymphocyte growth correlates well with the histologic rejection grade of the endomyocardial biopsy.[12] Biopsies with greater cellular infiltrates yield more lymphocyte cultures. Similar observations have been made with liver[13] and kidney transplant patients.[14,15] A similar relationship between biopsy growth and histologic grade of rejection in cardiac recipients on FK 506[16] has been established. Moreover, the frequency of biopsy growth was lower in FK 506-treated patients than that of patients on CyA-based immunosuppressive therapy. These results are consistent with the previously reported clinical experience of a lower incidence of cellular rejection in heart transplant recipients receiving FK 506 therapy.[17] A significant correlation was also observed between a positive biopsy growth assay and histologic rejection in FK 506-treated liver transplant recipients.[18] A significant number of histologically negative biopsies yield lymphocyte cultures. Studies on heart transplant patients have demonstrated that such growth correlates with a higher risk of subsequent rejection.[12] Since biopsy growth is a sensitive indicator of rejection, we have analyzed its relationship to plasma FK 506 levels in liver[18] and heart transplant patients.[16] The results, summarized in Table 1,

Table 1. Correlation Between FK 506 Plasma Levels and Biopsy Growth of Liver and Heart Transplant Recipients

Transplant Recipients	FK 506 Assay	FK 506 Plasma Levels (nmol/L) Biopsy Growth	
		Positive	Negative
Liver	ELISA	1.48 ± 1.45	4.06 ± 5.8
	Bioassay	0.30 ± 0.36	0.90 ± 0.63
Heart	ELISA	1.27 ± 0.13	2.50 ± 0.90

demonstrate that biopsy growth correlates with significantly lower FK 506 levels measured by ELISA, and in the liver transplant patients by bioassay as well. They are concordant with the clinically derived conclusions about therapeutic trough levels of plasma FK 506 in the 0.8 to 2 ng/mL range.[5]

IMMUNOSUPPRESSIVE DRUG SENSITIVITY OF LYMPHOCYTES PROPAGATED FROM ALLOGRAFT BIOPSIES

Transplant biopsy cultures also provide opportunities to study lymphocyte sensitivity to immunosuppressive drugs. Up to 100-fold differences have been observed in the sensitivity of heart biopsy cultured lymphocytes and alloreactive T-cell clones from different individuals to CyA.[19] In contrast, lymphocytes from different individuals exhibit a narrow sensitivity range (up to 10-fold) to FK 506. Transplant biopsy cultured lymphocytes are also useful to evaluate the immunosuppressive activity of drug metabolites.[19]

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