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Practical Aspects of FK 506 Analysis (Pittsburgh Experience)

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FK 506 is a potent immunosuppressive compound.[1] However, like cyclosporine (CyA) it has certain side effects, such as neurotoxicity and nephrotoxicity. This fact is very well documented in a recent paper by Abu-Elmagd et al.[2] In this paper, the authors have indicated that patients who continued to have bad liver functions after transplantation have high FK 506 concentrations and correspondingly increased nephrotoxicity that appears to remain for a longer period of time in spite of receiving lower dosages. There is also wide variations in the pharmacokinetics of FK 506. This means that for a given dose of FK 506, there is a wide variation in plasma concentrations. Additionally, FK 506, like CyA, exhibits a narrow therapeutic index.

At the University of Pittsburgh Medical Center, we have been analyzing plasma FK 506 levels for the past 3 years. Over the past 18 months, our workload has steadily increased to 5,000 patient analyses per month indicating that at our institution this drug is used very extensively. During this period we have gained tremendous amount of experience and expertise in performing the analysis of this drug.

Detailed procedures of plasma analysis of FK 506 by enzyme-linked immunoassay (ELISA) has been already published.[3] In our laboratory, use of the C-18 Sep-Pak column to free the drug from proteins and subsequent use of methanol to elute this drug from the column has been successfully employed. Stability studies of this drug in whole blood indicated that the drug is stable in blood for up to 4 days. This information is very important since out of 1,600 patients who currently receive this drug, 80% are outpatients who send us blood specimens by overnight mail.

The maximum partitioning of the drug into plasma was achieved by incubating the blood at 37°C for 1 hour. Plasma separated at 37°C normally has 30% to 40% higher concentrations when compared with plasma separated at room temperature. Modification of the original protocol from overnight incubation at 4°C to 2 hours incubation at room temperature showed a very good correlation (slope 0.92, correlation coefficient 0.89). Use of the 2-hour incubation method has enabled us to provide FK 506 results on the same day for critically ill patients, thus resulting in better patient care.

All transplant patients develop certain infections, and as a result, are treated with drugs such as erythromycin, gentamycin, and vancomycin. Since these drugs are administered in the presence of FK 506, their interference with FK 506 monoclonal antibody (MAb) was examined.

Table 1. Summary of FK 506 Analysis

Sample Analyzed	Plasma	Blood
Separation temperature	37°C	—
Storage conditions	−70°C	−70°C
Extraction method	Solid phase	Solid phase
Turnaround time		
ICU patients	8 h	8 h
All other patients	24 h	24 h

Abbreviation: ICU, intensive care unit.

We did not see any cross-reactivity even in the presence of peak concentrations of these drugs. Other immunosuppressants such as CyA (up to 2 μg/mL) and rapamycin (up to 20 ng/mL) also did not show any cross-reactivity with the MAb. Additionally, the presence of hemoglobin (up to 1.2 g/dL) and bilirubin (up to 25 mg/dL) did not show any interference in the analysis of FK 506 in plasma. Since the majority (75% to 80%) of this drug appears to bind to red blood cells, we developed a whole blood ELISA assay to measure FK 506 using solid phase extraction. The whole blood trough FK 506 concentrations are 8 to 10 times higher in comparison with the corresponding plasma concentrations.

Tables 1 and 2 summarize FK 506 analysis, sensitivity, linearity, and precision data for both plasma and whole blood analyses of FK 506.

Preliminary studies in our laboratory have shown that high-performance liquid chromatography (HPLC) alone cannot be used to measure the parent drug and its metabolites. At the present time combination of HPLC-ELISA is one way to quantitate parent FK 506 in biologic fluids. We have recently developed an HPLC method with a gradient system to obtain the fractions of parent drug as well as possible metabolites and to further quantitate them by ELISA. It appears that the unchanged FK 506 is a major component in plasma that reacts with the MAb. Analysis of FK 506 by HPLC-mass spectrophotometry (HPLC-MS) is being currently investigated at our institution. However,

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Table 2. Summary of All Parameters

Parameter	Plasma		Blood	
Sample volume	100 μL		25 μL	
Minimum detectable limit	0.1 ng/mL		0.8 ng/mL	
Standard curve range	0.1–10.0 ng/mL		0.8–80.0 ng/mL	
Control ranges	ng/mL		ng/mL	
Level 1	1.0–2.2		8.5–12.7	
Level 2	2.3–3.7		13.7–17.3	
Level 3	4.2–6.8		21.4–37.1	
Intraday variation (n = 10)	Mean*	C.V.†	Mean*	C.V.†
Level 1	1.5	4.2	10.6	10.0
Level 2	2.7	5.5	15.5	10.2
Level 3	5.1	7.1	29.3	13.4
Interday variation (n = 50)	Mean*	C.V.†	Mean*	C.V.†
Level 1	1.4	17.0	9.5	14.0
Level 2	2.9	14.4	14.0	11.1
Level 3	5.7	12.0	28.0	13.0

Abbreviation: CV, coefficient of variation.
*Results are in ng/mL.
†Results are in percent.

at the present time, in routine clinical practice, ELISA using solid phase extraction, is the most reliable and practical way to measure this drug in plasma as well as in whole blood.

Future studies have to be carried out to correlate blood and plasma FK 506 concentrations measured by ELISA, HPLC-ELISA, and HPLC-MS with bioassay, immunologic response measurement, and toxicity before a final decision is made as to the choice of the biologic fluid that should be used in routine clinical monitoring of FK 506. It is essential that in any method developed, one should know the nature of the compound that is being measured (parent drug and/or metabolites) and that the method be simple and provide rapid turn around time to be of practical value in treating patients.

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REFERENCES

1. Starzl T, Abu-Elmagd K, Tzakis A, et al: Transplant Proc 22:914, 1991

2. Abu-Elmagd K, Fung J, Alessiani M, et al: Transplantation 52:71, 1991

3. Tamura K, Kobayashi M, Hashimato K, et al: Transplant Proc 19:23, 1987