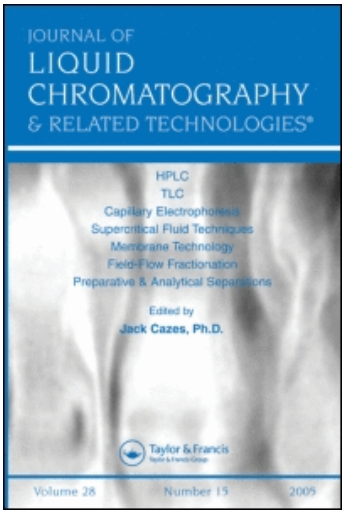

Journal of Liquid Chromatography & Related Technologies

Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713597273

Therapeutic Monitoring of Tacrolimus (FK 506) Using Liquid Chromatography. A Review

Amitava Dasgupta[a]; Timothy G. Timmerman[a]
[a] Department of Pathology, University of New Mexico, School of Medicine, Albuquerque, New Mexico

To cite this Article Dasgupta, Amitava and Timmerman, Timothy G.(1995) 'Therapeutic Monitoring of Tacrolimus (FK 506) Using Liquid Chromatography. A Review', Journal of Liquid Chromatography & Related Technologies, 18: 20, 4029 — 4038
To link to this Article: DOI: 10.1080/10826079508013743
URL: http://dx.doi.org/10.1080/10826079508013743

JOURNAL OF LIQUID CHROMATOGRAPHY, 18(20), 4029-4038 (1995)

THERAPEUTIC MONITORING OF TACROLIMUS (FK 506) USING LIQUID CHROMATOGRAPHY. A REVIEW

AMITAVA DASGUPTA AND TIMOTHY G. TIMMERMAN
Department of Pathology
University of New Mexico School of Medicine
Albuquerque, New Mexico 87106

Abstract

Tacrolimus (FK 506) is an macrolide immunosuppressive agent which is widely used for management of transplant patients. The drug is potent at concentrations well below the concentration of cyclosporine for patient management, but like cyclosporine, FK 506 also shows side effects such as nephrotoxicity and neurotoxicity. Because FK 506 exhibits wide variation in pharmacokinetics and a narrow therapeutic range, therapeutic monitoring of the drug is essential. Concentration of FK 506 can be monitored by high performance liquid chromatography (HPLC), Enzyme Linked Immunoassay (ELISA), HPLC-ELISA, HPLC-Mass Spectrometry and Fluorescence Polarization (FP) Immunoassay.

FK 506, The Discovery

The immunosuppressive agent Tacrolimus (FK 506, Prograf, Fujisawa Pharmaceutical Company, Osaka, Japan) was isolated from fermentation broth of Streptomyces tsukubaensis in 1984 (1,2). The stain was named as tsukubaensis because the stain was isolated from a soil sample of Tsukuba, Ibaraki, Prefecture, Japan. The drug was recently named as tacrolimus. The drug was demonstrated to posses

immunosuppressant properties by inhibiting interleukin-2 (IL-2) production in vitro and also inhibited the response of mixed lymphocyte culture at concentrations even 100 times lower than the widely used immunosuppressant, cyclosporine (3). In 1989, the drug was tested on a liver transplant recipient who did not respond to the immunosuppressant therapy using cyclosporine (4). The investigators of the University of Pittsburgh found FK 506, to be a very effective drug for immunosuppression with relatively few side effects, but the investigators in United Kingdom found the drug very toxic for management of transplant patients. The apparent discrepancy was found later due to lack of proper knowledge of pharmacokinetic parameters of the drug as well as lack of proper therapeutic drug monitoring. Large clinical trial of FK 506 was started in 1989 for patients undergoing chronic liver allograft rejection. Currently, the drug is approved by Federal Drug Administration (FDA) for management of patients receiving liver allografts.

Following the first international congress on FK-506, the Pittsburgh group reported 10 % improvement in patient survival, 16 % improvement of graft survival, and 21% lower rejection rate in liver transplant patients with replacement of cyclosporine by FK 506 (5). In addition, FK 506 can reverse a rejection episode where cyclosporine is ineffective (6). In kidney transplant patients, use of FK 506 as an immunosuppressant, eliminated the need of using prednisone or antihypertensive agents. Fk 506 also showed promises for use in heart transplant patients (7). The immunosuppressant activity was found to be effective as concentrations at least ten times lower than cyclosporine.

Chemical Structure and Pharmacology

FK 506 is a 23 membered macrolide lactone which is neutral, hydrophobic and crystallizes as colorless prisms. The molecular weight of the compound is 803. The structure includes a hemiketal function and an alpha, beta diketonamide group. Unlike cyclosporine, FK 506 is soluble in alcohol. The drug is also soluble in acetone, ethyl acetate, acetonitrile, dichloromethane, chloroform etc but insoluble in water. The drug is present in two conformational forms in organic solvent and the relative amounts of

each conformer depends on the temperature. The drug is quite stable at room temperature for many months in crystalline form, but is less stable in solution (1, 8,9). The structure of FK 506 is entirely different from the structure of cyclosporine which is cyclic peptide containing 11 amino acids. However, FK 506 has structural similarity with potential immunosuppressant rapamycin, and both contains pipecolic acid moiety. However, the mechanism of action of FK 506 is different from rapamycin.

FK 506 can be administered as oral dose intravenous injection. The oral bioavailability of the drug is variable (5-67%) due to poor and erratical absorption. However, unlike cyclosporine, absorption of FK 506 is less dependent on the availability of bile. After absorption, the drug is rapidly distributed outside plasma compartment and the volume of distribution ranges from 5 to 65 L/Kg (10). In plasma, the drug is largely bound to alpha-1 acid glycoprotein (11) and in whole blood FK 506 is mainly associated with erythrocytes. The half life of the drug can vary between 3.5 to 40 h with a mean of 11.3 h

The rapid clearance of FK 506 compared to cyclosporine is related to liver not renal function. In rat model, FK 506 was found to distribute more rapidly than cyclosporine. Coadministration of erythromycin causes accumulation of parent drug while administration of steroids lead to accumulation of inactive metabolites of FK 506.

FK 506 is metabolized by liver and small intestinal microsomes containing cytochrome P-450 3A and the fast pass metabolism by liver may be important in the disposition of the drug (12). Following metabolism, approximately 95 % of the drug is eliminated by biliary route mainly as metabolites and about 5% are excreted in urine unchanged. The drug is extensively metabolized to several metabolites including O-demethylated, hydroxylated, O-demethylated hydroxylated and dihydrodiol metabolites (13-14). Recently, the 15-demethylated, 13-demethylated and double demethylated metabolite from erythromycin-induced human liver microsome has been reported (15-16). Georges et al also recently reported the isolation of 15 desmethyl FK 506 and 15, 31-desmethyl FK 506 from human liver microsomes which retain in vitro immunosuppressive activity, using

fast atom bombardment mass spectrometry and nuclear magnetic resonance spectrometry (17). FK 506 should be used with caution in patients receiving microsomal enzyme inducer or inhibitors. FK 506 itself also inhibits the activity of cytochrome P-450 dependent drug metabolism.

Mechanism of action of FK 506

Although the precise molecular action is not completely understood, FK 506 has similar immunosuppressive property of cyclosporine. However, FK 506 can suppress murine or human mixed lymphocyte reactivity and the generation of cytotoxic T cells at concentrations 100 times lower than that of cyclosporine. FK 506 inhibits early T-cell activation events that are required for lymphokine gene expression (18). Like cyclosporine, FK 506 also selectively blocks calcium dependent intracellular signaling events in others, possibly related signal transduction pathways. FK 506 however, binds to cytosolic receptors which are different from cyclophilin A, the major receptor which binds cyclosporine. FK 506 binds to a 12 k-Da cytosolic FK 506 binding protein termed as FKBP 12. The FKBP 12.FK 506 complex binds specifically to the calcium and calmodulin dependent serine and threonine phosphatase, calcineurin and inhibits its phosphatase activity in vitro. Recently, Wiederrecht et al using gel filtration technique identified a novel FK 506 binding high molecular weight protein with 110 k-Da molecular weight (19).

Toxicity of FK 506

Since both FK 506 and cyclosporine inhibits a series of isomerase enzymes, there are some similarities in their side effects. However, FK 506 is free from some side effects of cyclosporine namely, gingival hyperplasia and hirsutism (20-21). However, FK 506 like cyclosporine can cause nephrotoxicity. FK 506 decreases glomerular filtration rate and renal blood flow but increases renal vascular resistance. The nephrotoxicity may be related to an increased production of thromboxane A 2 production in the renal parenchyma (22). FK 506 also can cause hypertension, but the incidence appears to be 50 % less than that experienced with cyclosporine. FK 506 also has a diabetogenic effect probably due to

a change in the islet cells's response to hyperglycemia and a change in peripheral sensitivity of insulin. The incidence of major neurological side effect is low (5%) with FK 506 and most of them occur during the first month following liver transplant (23). According to the Pittsburgh study, the patterns and timing of opportunistic infections after surgery is similar under FK 506 and cyclosporine therapy, occurring early in the post transplant stage (24).

Therapeutic Drug Monitoring of FK 506

Therapeutic drug monitoring of FK 506 presents an analytical challenge because the recommended trough plasma levels are 0.5-2.0 ng/mL for clinically stable liver and kidney transplant recipients. Another major problem in therapeutic monitoring of FK 506 is that like cyclosporine, erythrocytes serves as a large reservoir for FK 506. The exchange of drug between erythrocytes and plasma is rapid and temperature dependent. Therefore plasma assay requires prior to separation from red blood cells, the sample should be equilibrated at least for 1 h at 37°C (25). At 37°C, the plasma levels of FK 506 are 30-40% higher than those found at room temperature.

The therapeutic range of FK 506 is still under study. They depend on the temperature at which the plasma was separated from blood cells, the extraction protocol as well as the assay techniques. A therapeutic range of 0.2-0.8 ng/mL was suggested for plasma if the separation of plasma from blood cell was achieved at room temperature, but a therapeutic range of 0.5-2.0 ng/mL was also recommended if plasma was separated from blood cells at 37°C. The concentration of FK 506 measured in whole blood was substantially higher than the concentration measured in plasma. A therapeutic range of 2.5-14 ng/mL was recommended for whole blood FK 506 concentration using ELISA assay following liquid/liquid extraction while a therapeutic range of 4.0-20 ng/mL has been recommended for Abbot's IMx assay for FK 506 (26).

HPLC in monitoring FK 506 concentration

FK 506's absorption at 192 nm allows HPLC isolation and U.V. detection only for pharmaceutical application where sensitivities

around 50-100 ng/ mL is acceptable but not for monitoring FK 506 concentration in plasma or whole blood. Takada et al determined FK 506 concentrations in rat serum using HPLC with chemiluminescence determination. The dansyl hydrazine reagent reacts with carbonyl group of FK 506 to form a fluorescent product with an excitation maximum at 350 nm and an emission maximum at 510 nm (27). The authors extracted FK 506 from 100 μL of rat plasma using ethyl acetate and derivatized FK 506 with dansyl hydrazine reagent. The excess derivatizing reagent was destroyed with sodium pyruvate solution and the initial clean up of sample was achieved with Sep-Pak cartridge and using 70 % and 80% methanol as eluting solvent. The HPLC procedure involves column switching technique using two different mobile phases. The mobile phase one contains methanol/water (70:30 by vol) and mobile phase II contains methanol/water (90:10 by vol). Both pre column and HPLC column used were derivatized silica (C-18 reverse phase). The lower end of sensitivity for the assay was 5 ng/mL of FK 506 concentration in plasma.

Wong et al described a supercritical fluid chromatography and HPLC of cyclosporine and FK 506. Using a Bondpack C-18 column at 70°C and acetonitrile/water (80:20 by vol) as a mobile phase, the two more polar tautomers of FK 506 eluted at 4 min as one peak while FK 506 showed a retention time of 5 min. The detection wavelength was 202 nm. However, in supercritical fluid chromatography (biphenyl column and pressure program from 100 to 300 atmosphere at 10 atmosphere/min, FID detection), the tautomers of FK 506 eluted (17.75 min) after FK 506 (17.48 min) (28).

HPLC - ELISA in Monitoring FK 506 Concentration

Friob et al described a combined HPLC-ElISA assay for therapeutic monitoring of FK 506 in transplant patients. The authors used 200 μL of serum for solid phase extraction and after drying, reconstituted the residue with 30 μL of methanol for HPLC injection. The HPLC system used a reverse phase Micro-Pak MCH-5, 15 cm column (heated at 72°C) and an isocratic solvent system of acetonitrile/water (71:29 by vol). The elution of peaks were monitored at 212 nm. The FK 506 tautomers eluted at 4.6 and 5.2 min with a proportion of peak areas of 1:9. For the ELISA assay, C 18

solid phase Bond-Elute columns were used to extract FK 506. The sensitivity limit reached as low as 0.1 ng/mL (29).

Warty et compared solid phase extraction followed by ELISA assay with liquid-liquid extraction, HPLC separation and ELISA assay for determining concentrations of FK 506 in plasma (30). The solid phase extraction using C-18 Sep-Pak cartridge required 100 μL of plasma (30). After prewashing cartridge with 4 % acetic acid, plasma sample was applied and FK 506 was extracted with methanol and analyzed by ELISA. The authors also described a liquid-liquid extraction of FK 506 from 100 μL of plasma with dichloromethane after initial acidification of plasma with 1 mL of 0.1 N hydrochloric acid. After extraction, excess solvent was evaporated and the dry residue was reconstituted with methanol for injection into HPLC. The HPLC column was a 3.9 mm X 15.0 cm analytical column filled with μBond Pak C-18. The column temperature was set at 60°C and the mobile phase composition was methanol/water (80:20 by vol), acidified to pH 6.0 with hydrochloric acid. The flow rate was 0.8 mL/min. The detection was achieved with a photodiode array detector at 214 nm. The retention time of FK 506 was 4.8 min. Authors collected two different fractions (0-3.6 min and 3.6-6.0 min) which contained 99% of all FK 506. Both fractions were evaporated and residue analyzed for FK 506 using ELISA. Authors found no difference in FK 506 concentrations obtained by solid phase extraction and ELISA versus liquid-liquid extraction, HOLC and ELISA. However, solid phase extraction and ELISA showed higher FK 506 levels than liquid-liquid extraction and HPLC only in patients with abnormal liver function. This may be due to cross reactivity of FK 506 metabolites with ELISA.

HPLC-Mass Spectrometry for FK 506 Monitoring

Christians et al described a HPLC-mass spectrometry assay for FK 506 using a synthetic internal standard, 32-O-acetyl FK 506 (31). Internal standard was synthesized by incubating FK 506 with acetic anhydride for 2 h at 75°C. After solid phase extraction of FK 506 and internal standard (5 ng) from 1 mL of whole blood or plasma using solid phase extraction, the parent drug and the metabolites were separated by a HPLC system using a reverse phase C-8 column (100 X 4 mm, particle size 3 μm). The separation was achieved with

solvent gradient using analysis time 0 min: 60% acetonitrile, analysis time 8.1 min: 95% acetonitrile. The flow rate was 0.3 mL/min. The retention time of FK 506 was 8.5 min while that of the internal standard was 10.5 min. For mass spectral analysis, the authors used negative chemical ionization with methane or butane as reactant gas. The temperature of mass spectrum source was 250°C while the temperature of quadruple was 120°C. The mass spectrum was run in a selected ion mode monitoring m/z 776, 790, 792, 804, 808, 836 and 834. The authors reported a limit of detection of 25 pg at a signal to noise ratio of 1:8 and the assay was linear from 25 pg to 50 ng. The between run precision was 10.5% at 5 ng level while the within run precisions were 4.7% and 7.7% respectively at 3 and 10 ng levels. In blood sample, FK 506 and a metabolite at 790 amu was regularly found. However, in urine, FK 506, and other metabolites at 776 amu (double demethylated), 790 (double demethylated) and 792 (double demethylated and hydroxylated) were also found. In HPLC with U.V. detection, several FK 506 metabolites can not be separated. The authors by using LC/MS can quantitate individual metabolites.

Immunoassays for monitoring FK 506 concentrations

Fuijisawa has described an enzyme linked immunoassay (ELISA) methodology to measure FK 506 concentrations. The assay used ELISA plates coated with anti FK 506 antibody, an FK 506 horseradish peroxidase conjugate that competes with free FK 506 and an appropriate substrate for peroxidase. However, ELISA is a semiautomated technique. Recently, Grenier et al described an automated assay for measuring FK 506 concentrations in whole blood using IMx analyzer (Abbott Laboratories, Abbott Park, IL). The FK 506 assay for the IMx analyzer utilizes four reagents: a precipitation reagent to extract FK 506 from whole blood, a capture reagent consisting of latex microparticles to which FK 506 antibody was covalently immobilized, an FK 506 alkaline phosphatase conjugate reagent and an enzyme substrate reagent consisting of 4-methylumbelliferyl phosphate. The sensitivity of the assay is 3.3 ng/mL while precision analysis showed CVs of 11.8, 9.6 and 8.1% at FK 506 levels of 15, 25 and 65 ng/mL (32).

References

1. T. Kino, H. Hataanaka, M. Hashimoto et al. J Antibiot 40: 1249-1255 (1987)
2. T. Kino, H. Hatanaka, S. Miyata et al. J Antibiot 40: 1256-1265 (1987).
3. T. Ochiai , K. Nakajima, M. Naqgata M. Transplant Proc 19: 1284-1286 (1987)
4. T. Starzl, S. Todo, J. Fung, A. Demetris, R. Venkataramana, Jain Lancet 2: 1000-1004 (1989)
5. J. Fung, A. Tzakis, S. Todo. Presented at the annual meeting of American Society for Transplant Surgeons, Chicaho, 1991.
6. J. Fung, S. Todo, A. Tzakis A. et al Transplant Proc 23: 1902-1905 (1991)
7. R. Shapiro, M. Jordan, V. Scantlebury et al. FK 506 in kidney transplantation. Presented at first international congress on FK 506, Pittsburgh, 1991.
8. H. Tanaka, A. Kuroda, H. Marusawa H. J Am Chem Soc 109: 5031-5033 (1987)
9. T. Honbo, M. Kobayashi, K. Hane, T. Hata et al Transplant Proc 19 (Suppl) 17-22 (1991)
10. R. Vankataramanan, A. Jain, VS Warty et al. Transplant Proc 23: 2736-2740 (1991)
11. R. Vankataramanan, A. Jain, E. Cadoff et al. Transplant Proc 22 (Suppl 1) 52-56 (1990)
12. M. Sattler, FP. Guengerich, C. Yun, U. Christians, KF. Sewing. Drug Metab Dispos 20: 753-761 (1992)
13. G. Lhoest, P. Wallemacq, RK. Verbeeck. Pharm Acta Helv 67: 302-306 (1991)
14. G. Lhoest, N. Maton, RK. Verbeeck. Drug Metab Dispos 21: 850-854 (1993)
15. G. Lhoest, N. Maton, A. Laurent. Pharma Acta Helv 68: 35-41 (1993).
16. SH, Vicent, BV. Karanam, SK. Painter, SHL. Chiu. 294: 454-460 (1992)
17. G. Lhoest, N. Maton, D. Latinne, A. Laurent A, RK. Verbeeck. Clin Chem 40: 740-744 (1994)
18. JE. Kay, CR. Benzie, MR. Goodier, CJ. Wick, EA. Doe. 67: 473-477 (1989)

19. G. Weiderrecht, S. Hung, HK. Chan, A. Marcy, M. Martin, J. Calaycay, D. Boulton, N. Sigal, RL. Kincaid, S. Siekierka. J Biol Chem 267; 21753-21760 (1992).
20. SL. Schreiber. Science 251; 283-287 (1991)
21. R. Reding, J. DeVille, E. Sokal. Transplant Proc 23; 3002-3004 (1991).
22. K. Yamada, Y. Sugisaki, S. Suzuki, M. Akimoto, H. Amemiya, N. Yamanaka. Transplant Int 5 (Supply); S564-S567 (1992).
23. BH. Eidelman, K. Abu-Elmagd, J. Wilson et al. Transplant Proc 23; 3171-3172 (1991).
24. M. Alessiani, S. Kusne, S. Martin et al. Transplant Proc 23; 1501-1503 (1991).
25. Chou D. Anal Chem. 65; 412R-415 R (1993).
26. P.E. Wallemacq, R. Reding. Clin Chem 39; 2219-2228 (1993).
27. K. Takada, M. Oh-Hashi, H. Yoshikawa, S. Muranishi, H. Tanaka. J Chromatogr 530; 212-218 (1990).
28. SHY. Wong, B. Ghodgaonkar, P. Fong, B. Campbell, JF Burdick, F. Boctor. J Liquid Chromatogr 17(10); 2093-2109 (1994).
29. MC. Friob, HD. Latinne, G. Lhoest, JB Otte, PE. Wallemacq. Transplant Proc 23; 2750-2752 (1991).
30. V. Warty, S. Zuckerman, R. Venkataramanan, J. Lever, J. Fung, T. Starzl. Ther Drug Monit 15; 204-208 (1993).
31. U. Christians, F. Braun, N. Kosian, M. Schmidt, HM. Schiebel, L. Ernst, C. Kruse, M. Winkler, I. Holze, A. Linck, K. Sewing. Transplant Proc 23; 2741-2744, (1991).
32. FC. Grenier, J. Luczkiw, M. Bergman, S. Lunetta, M. Morrison, D. Blonski, S. Shoemaker, M. Kobayashi. Transplant Proc 23; 2748-2749 (1991).

Received: April 14, 1995
Accepted: May 30, 1995