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Pharmacokinetics of FK 506: Preclinical and Clinical Studies

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FK 506 is a macrolide with potent immunosuppressive effects. Its immunosuppressive property has been documented in both in vitro[1] lymphocyte proliferation studies and in vivo studies. FK 506 treatment significantly prolongs graft survival in rats receiving heterotopic heart transplants, dogs receiving kidneys or livers, and baboons receiving kidney transplants.[2] Currently, FK 506 is undergoing clinical trials at the University of Pittsburgh. This document provides a summary of the biopharmaceutical aspects and the pharmacokinetics of the drug in animals and humans.

CHEMICAL AND PHYSICOCHEMICAL PROPERTIES

FK 506 belongs to a group of chemicals referred to as the macrolides. Macrolides are macrocyclic lactones containing keto and hydroxyl groups. Other macrolides that are presently known include erythromycin, oleandomycin, spiromycin, carbomycin, josamycin, leucomycin, tylosin, and rapamycin. In addition to FK 506, rapamycin has also been reported to possess some immunosuppressive activity.

FK 506 has a molecular weight of 822. It is highly lipophilic in nature. It is very soluble in methanol, chloroform, acetone, ethyl acetate, ethanol, and propylene glycol. It is moderately soluble in polyethylene glycol and ether, slightly soluble in olive oil and glycerin, and poorly soluble in water and hexane.[3] FK 506 is stable in solid state and in the presence of very dilute HCl. It is degraded into multiple components in the presence of 6 N or concentrated HCl. Methanolic solution of FK 506 is stable for several months at −20°C.

DOSAGE FORM OF FK 506

Preclinical pharmacokinetic studies have been carried out with intravenous, oral, and intramuscular dosage forms of FK 506. Clinical studies have used the intravenous and oral dosage forms of FK 506.

The intravenous dosage form of FK is available as a 10 mg/ml solution. This solution contains FK 506, polyoxyethylated hydrogenated castor oil (HCO-60, a surfactant), and alcohol. The intravenous preparation must be diluted with saline or dextrose and administered as an infusion for 1 or 2 hours in humans. Diluted intravenous FK 506 solution has been used for rapid infusion (less than 30 seconds) in animal studies. Intravenous FK 506 in saline or dextrose is stable; more than 90% of the dose is available to patients from a dextrose solution stored in glass containers for 24 hours, while greater than 10% of the drug is adsorbed onto plastic minibags within 6 hours.

The oral dosage form is a 20% solid dispersion of FK 506 in hydroxy propyl methyl cellulose and HCO-60. This dispersion is administered in a hard gelatin capsule. The intramuscular dosage form used in animal studies contains 27% of FK 506 in mannitol and HCO-60. This is normally suspended in water or saline and is administered as a suspension.

DRUG ANALYSIS

In clinical studies and in studies involving dogs and rats, FK 506 concentrations were measured by a monoclonal antibody-based ELISA as described elsewhere in this issue.[4] FK 506 concentrations in baboon studies and in rat tissues were measured by a polyclonal antibody-based ELISA.

PRECLINICAL STUDIES

Intravenous Kinetics and Absorption

Dogs. In the studies reported here, the plasma is separated from blood sample at 37°C. Following intravenous administration (0.2 mg/kg), the concentration of FK 506 declines very rapidly (Fig 1). This corresponds to the distribution of FK 506 outside the vascular system. Once postdistribution equilibrium is established, the FK 506 concentrations decline slowly over the rest of the dosing interval. This primarily indicates elimination of the drug from the central compartment and redistribution of the drug from the extravascular compartments. The mean terminal disposition half-life of FK 506 is 10.3 hours, with a range of 9.0 to 13.2 hours. The mean plasma clearance is 2,325 ml/min, with a range of 1,943 to 3,125 ml/min. This value is greater than the expected hepatic blood flow in dogs, suggesting possible extrahepatic metabolism of the drug. The high clearance also indicates that following oral administration, FK 506 is expected to undergo significant first-pass metabolism.

Following oral administration (1 mg/kg), the drug is rapidly absorbed as indicated: a very short time is required (1 to 2 hours) for achieving peak plasma concentrations. Peak plasma concentrations range from 1.9 ng/ml to 4.9

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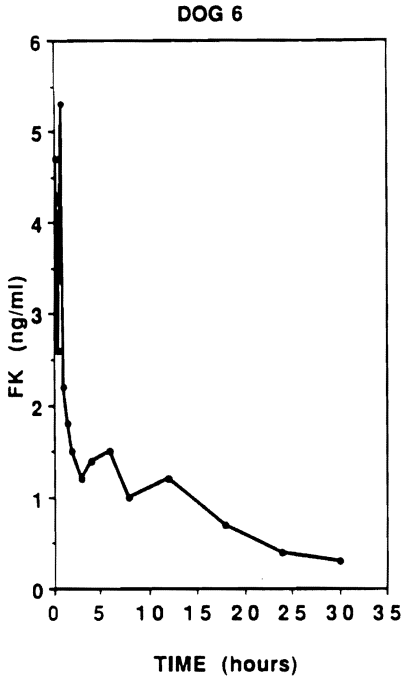

Fig 1. Plasma concentration versus time profile after intravenous administration of FK 506 (0.2 mg/kg) to dogs.

ng/ml, with a mean value of 2.8 ng/ml. The mean half-life is 7 hours, with a range of 5.6 to 7.9 hours. The absolute oral bioavailability is calculated to be 9%, with a range of 5 to 12%. In some dogs, secondary peaks are observed in the plasma concentration versus time curves. This is likely to be related to the poor solubility of FK 506 and the consequent precipitation, redissolution, and absorption of the drug after oral administration. The absence of any significant amount of the drug in the bile supports this hypothesis.

Following intramuscular administration, FK 506 is absorbed very slowly and erratically. Steady-state concentrations are reached in 4 to 12 hours and the concentrations are maintained for at least 24 hours.[5]

Baboons. In this study, the plasma is separated from blood sample at 24°C. Single oral dose administration of FK 506 (10 mg/kg) to baboons (n = 4) results in a peak plasma concentration of 8.1 + 1.0 ng/ml. These concentrations are reached in 3.8 ± 1.4 hours after oral administration. The half-life of FK is 9.6 ± 2.0 hours.

Rats. FK 506 is absorbed rapidly after oral administration to rats. The presence of large amounts of radioactivity in the alimentary canal of bile duct cannulated rats following oral administration of radioactive FK 506 indicates incomplete absorption of the drug from the gut (Internal Research Report, Fujisawa Pharmaceuticals Co Ltd, Osaka, Japan). The half-life of FK 506 in rats appears to be less than 3 hours.

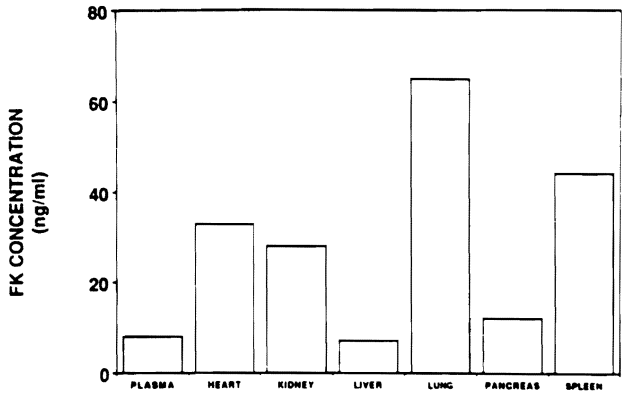

Fig 2. Tissue concentrations of FK 506 in rats after 14 days of intramuscular treatment (1.28 mg/kg/d).

Distribution

Distribution of FK 506 in various organs in the body is determined after intramuscular administration of 1.28 mg/kg for 14 days (Fig 2). This study indicates that the concentrations are in the following order: lungs > spleen > heart > kidney > pancreas > liver = plasma (24°C). The mean concentration in the lung tissue is nearly eight times that present in the plasma.

Elimination

The elimination of FK 506 after intravenous or oral administration has been studied in rats (Fig 3). Studies using radioactive FK 506 (^{14}C) indicate that about 11% of the radioactive material administered intravenously is excreted in the urine, while nearly 80% of the radioactivity is excreted in the bile and 8% is in the feces within 48 hours (Internal Research Report, Fujisawa Pharmaceutical Co Ltd). Following oral administration, about 4% of the radioactive material is excreted in urine, 34% is in the

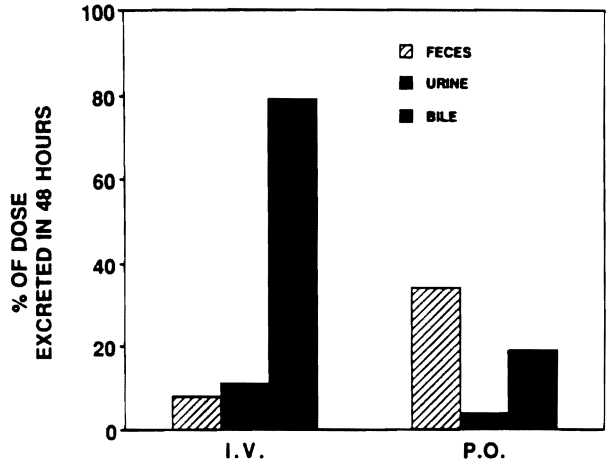

Fig 3. Percent of the dose excreted in urine, feces, and bile within 48 hours after intravenous and oral administration to bile duct cannulated rats.

feces, and 19% appears in the bile. Less than 1% of the dose is excreted in the urine or bile as unchanged drug within 48 hours in rats and in dogs, indicating complete metabolism of the drug.

Drug Interactions

Chronic intramuscular administration of FK 506 (1.28 mg/kg) decreases cytochrome P-450 concentrations (0.9 ± 0.92 to 0.68 ± 0.03 N mol/mg protein) and decreases the activity of ethylmorphine N-demethylase (19.3 ± 2.7 to 6.4 ± 1.8 N mol HCHO/min/mg protein) in male Wistar rats.[5] In Wistar rats, FK 506 (1 mg/kg/d for 4 days) increases the trough concentration of CyA after a single intravenous bolus dose of 4 mg/kg (test animals, 643 ± 135 ng/ml; control animals, 368 ± 99 ng/ml). These observations indicate that FK 506 may inhibit the metabolism of other coadministered drugs.

Drug Accumulation

Chronic administration of a drug more often than once in six half-lives is expected to result in accumulation of the drug. Based on trough concentrations and measurements of area under plasma concentration (AUC), it can be concluded that in baboons and dogs, FK 506 accumulates in the body after chronic oral administration (Internal Research Report, Fujisawa Pharmaceuticals Co Ltd).

Dose and Time Dependency in Kinetics

In dogs, the AUC values after a single oral dose increase in proportion to the dose administered, up to a dose of 3.2 mg/kg. At a dose of 4 mg/kg, there is a disproportionate increase in the AUC, indicating some nonlinearity in the kinetic processes involved.[5] In baboons, there is no evidence of any nonlinearity up to a single oral dose of 10 mg/kg (Internal Research Report, Fujisawa Pharmaceutical Co Ltd).

FK 506 kinetics do not change after chronic intramuscular treatment in baboons, as indicated by similar half-lives (9.6 ± 2 hours on day 1 and 9.5 ± 1 hour on day 28) and time to reach peak plasma concentrations (3.8 ± 1.4 hours on day 1 versus 3.3 ± 1.0 hour on day 28) after oral administration on day 1 and 28.

CLINICAL STUDIES

Intravenous Administration and Absorption

In all human kinetic studies, plasma is separated from blood samples at 37°C. Following intravenous infusion (0.15 mg/kg over 2 hours), FK 506 concentrations decline rapidly (Fig 4). Peak concentrations at the end of infusion range from 10 to 24 ng/ml. Once the distribution equilibrium is reached, the drug concentrations decline slowly over the next 24 hours. The half-life ranges from 5.5 to 16.6 hours, with a mean of 8.7 hours. The plasma clearance averages 143 L/h, ranging from 87 to 269 L/h. This indicates that FK 506 is a high-clearance drug. The mean volume of distribution is 1,342 L, suggesting extensive distribution of the drug in the body.

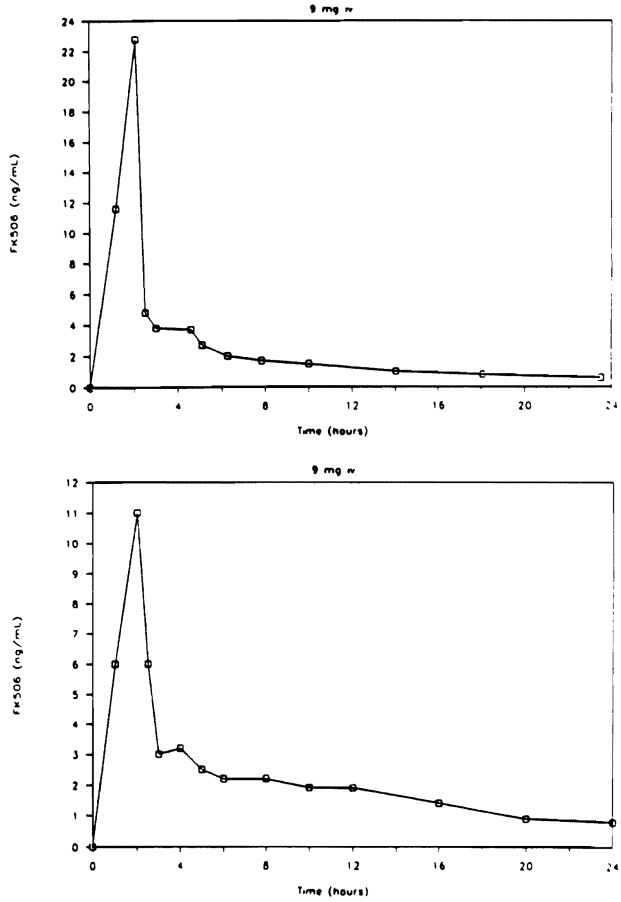

Fig 4. Plasma concentration of FK 506 versus time curve after intravenous infusion (9 mg over 2 hours) in two patients.

Following oral administration, FK 506 is poorly, erratically, and incompletely absorbed (Fig 5). In some patients, the drug seems to be absorbed continuously over most of the dosing interval. An oral dose of 0.15 mg/kg results in a peak plasma concentration of 0.4 to 3.7 ng/ml. The time to reach peak concentration varies from 1 to 4 hours. The mean bioavailability calculated is 27%.

Distribution

The large volume of distribution indicates extensive extravascular uptake of FK 506. Even though FK 506 concentrations have not been measured in any human tissues, based on animal studies, high concentrations of FK 506 are expected in lung, kidney, heart, and spleen. At a concentration of 250 ng/ml, the blood to plasma concentration of FK 506 is 1.5, indicating uptake into red blood cells. Within plasma, FK 506 primarily resides in the lipoprotein-deficient fraction. As shown in Table 1, only 24% of the FK 506 is taken up in the various lipoprotein fractions, as compared with nearly 77% in the case of CyA.

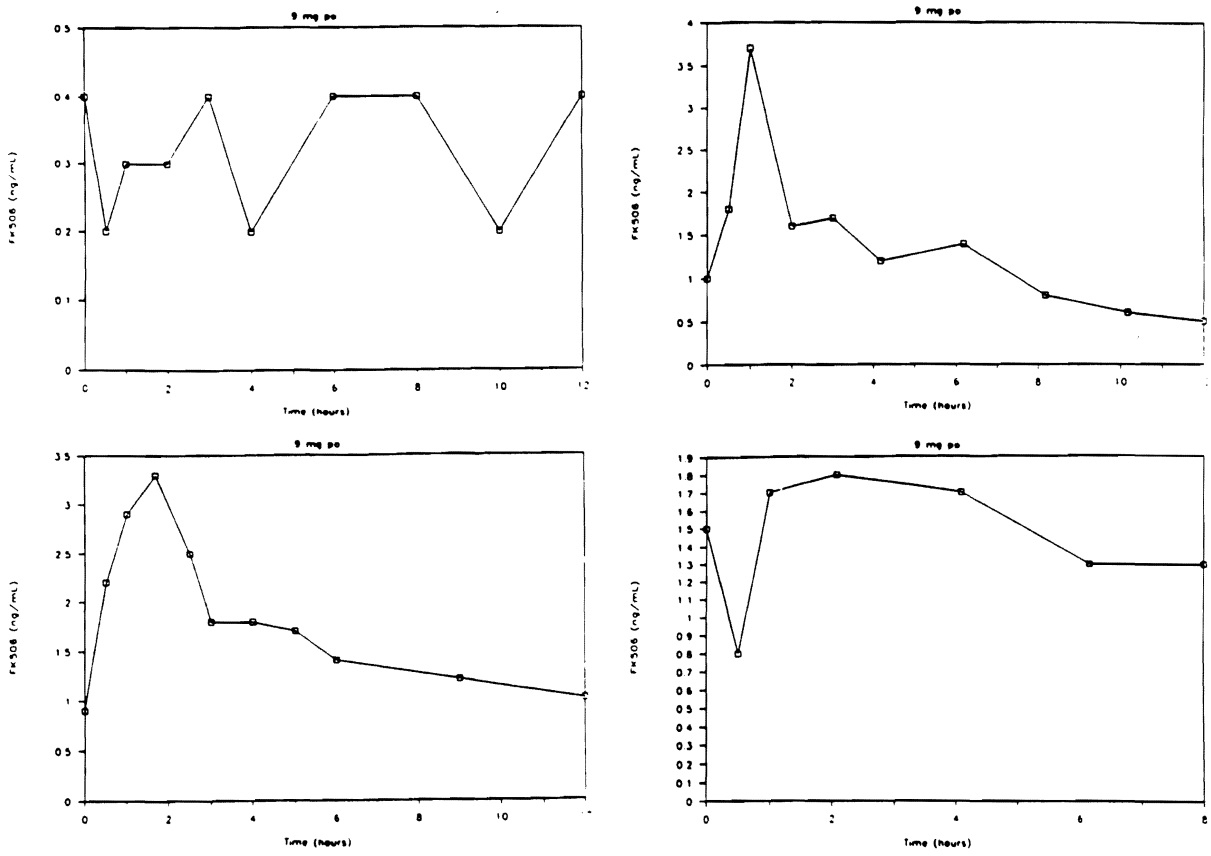

Fig 5. Plasma concentration of FK 506 versus time curve after oral administration of 9 mg of FK 506 to four patients.

The concentration of FK 506 in plasma is dependent on the temperature at which the blood is separated. The concentration in the plasma separated at 37°C is nearly twice that separated at 24°C (Fig 6). Attention must be paid to the proper sample preparation technique.

In one patient, simultaneous blood and cerebrospinal fluid samples were obtained, and it was found that when the plasma concentration was 3.3 ng/ml, the concentration in the cerebrospinal fluid was <0.1 ng/ml.

Elimination

Less than 1% of the intravenous or oral dose of FK 506 appears in the urine. This indicates the drug to be completely metabolized prior to elimination from the body. The concentration of FK 506 in dialysis fluid is less than the detection limits of the assay used. Since the drug is completely metabolized, it is highly lipid-soluble, has a high volume of distribution, and, with a molecular weight of 822, it is not expected to be dialyzable. These observations indicate that dosing regimen changes may not be necessary in patients with renal failure or in patients undergoing dialysis. However, the kinetics of FK 506 are expected to be altered in patients with liver dysfunction.

The metabolic pathways of FK 506 have not been

Table 1. Distribution of FK 506 in Plasma

	FK 506 (%)	CyA (%)
Very-low-density lipoprotein	1.9	10
Low-density lipoprotein	2.7	32
High-density lipoprotein	19.6	37
Lipoprotein-deficient fraction	76.1	23

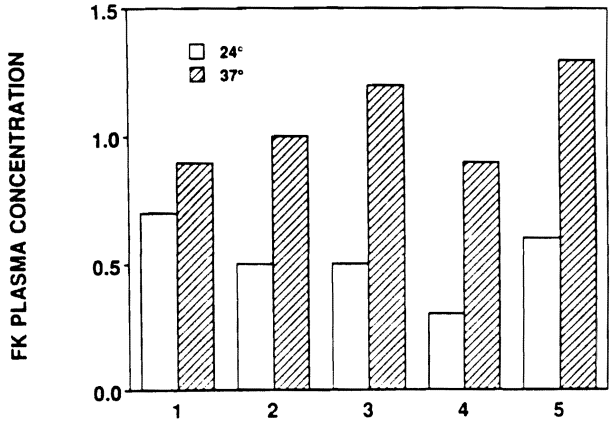

Fig 6. Temperature-dependent plasma FK 506 concentration in patients. □, Blood sample separated at 24°C; ▨, blood sample separated at 37°C.

completely characterized. It is expected to undergo O-demethylation and possible conjugation.

Dose and Time Dependency

Preliminary data indicate that at dose levels of 0.05 and 0.15 mg/kg IV, there is no dose dependency in the AUC in patients. Time dependency in FK 506 kinetics has not been completely characterized.

Drug Interaction

In patients receiving FK 506, the half-life of CyA is prolonged. The half-life of CyA in patients not receiving FK 506 is normally 6 to 15 hours. In patients on FK 506 who have a normal bilirubin concentration, the half-life of CyA after oral administration calculated from the concentrations based on fluorescent polarization immunoassay ranges from 26 to 74 hours. This indicates inhibition of CyA metabolism or potential alterations in CyA absorption by FK 506.

Of the 17 incidences of methylprednisolone treatments, FK 506 plasma concentrations increased on 10 occasions, decreased on 5 occasions, and did not change on 2 occasions. The potential effect of methylprednisolone on FK 506 kinetics needs further study.

Monitoring of FK 506

The pharmacokinetics of FK 506 are variable among patients, and the absorption of the drug after oral administration is variable and incomplete. This indicates the need for monitoring the plasma concentrations of FK 506 in patients to avoid potential rejection of the transplanted organ. Since the distribution of FK 506 in blood is temperature-dependent, plasma must be separated at carefully controlled temperatures. In our preliminary observations, the trough concentrations of FK 506 range from <0.1 ng/ml to nearly 5 ng/ml after an oral dose of 0.15 mg/kg/d. There is no specific side effect associated with higher trough concentrations. It appears that FK 506 may have a larger therapeutic index and, therefore, it may not be necessary to monitor the drug every day.

DISCUSSION

Preclinical

FK 506 is poorly absorbed after oral administration and, therefore, a higher oral dose is required for achieving similar plasma concentrations when compared with an intravenous dose. It is extensively distributed in various tissues, and concentrations exceeding plasma concentrations are observed in lung, spleen, heart, and kidney. FK 506 is completely metabolized prior to elimination from the body. Based on the half-life of FK 506, a dosing interval of 12 or 24 hours seems normally appropriate. FK 506 accumulates in the body after chronic dosing. However, the kinetics of FK 506 do not change after chronic oral treatment. At single oral doses below 3.2 mg/kg, the kinetics appear to be linear. FK 506 decreases the activity of hepatic drug metabolizing enzymes. Dosing regimen of some coadministered drugs may have to be reduced to prevent toxicity.

Table 2. Pharmacokinetic Properties of FK 506

Absorption
Rate: Rapid
Extent: 27%
Distribution
Extensive tissue distribution
Elimination
High-clearance drug
Completely metabolized
Less than 1% of the dose excreted in urine
Half-life: 8.7 hours
Drug interactions
FK 506 increases CyA concentration
Potential for inhibition of metabolism of other drugs

Clinical

The various pharmacokinetic properties of FK 506 are summarized in Table 2. FK 506 is poorly and incompletely absorbed after oral administration. The drug is extensively distributed in the body. FK 506 is a high-clearance drug and is eliminated by metabolism. From a pharmacokinetic basis, changes in FK 506 dosing regimen may not be necessary in patients with kidney failure and in patients on dialysis, but may be necessary in patients with hepatic dysfunction. Based on the half-life, a once- or twice-a-day regimen appears to be appropriate in humans. FK 506 increases CyA concentrations and may affect the concentrations of other coadministered drugs. Since FK 506 is primarily eliminated by metabolism, its kinetics may be affected by other drugs. Monitoring of FK 506 concentrations will help in optimizing therapy with this drug.

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