Pharmacokinetic parameters calculated from i.v. and oral single doses in rats.

Route	Dose (mg/kg)	Parameters	Whole Blood FK506	Whole Blood Radioactivity	Plasma FK506	Plasma Radioactivity
I.V.	1.0	t½ (h)	6.4	10.4	5.6	12.3
		AUC $_{0-\infty}$ (*)	313.7	966.0	228.0	860.0
		Vd (l/kg)	11.8	13.0	15.0	15.6
		Clearance (l/h x kg)	1.6	1.0	3.9	1.2
P.O.	1.0	t½ (h)	5.0	-	7.0	-
		AUC $_{0-\infty}$ (*)	39.1	-	10.9	-
		Tmax (h)	0.25	-	0.25	-
		Cmax (ng/ml)	6.7	-	2.9	-
		Bioavailability	12.5%	-	4.8%	-
P.O.	3.2	t½ (h)	6.2	-	6.3	-
		AUC $_{0-\infty}$ (*)	285.8	943.0	68.9	972.0
		Tmax (h)	1.0	2.0	1.0	2.0
		Cmax (ng/ml)	41.0	92.0	20.0	94.0
P.O.	10.0	t½ (h)	6.0	-	2.8	-
		AUC $_{0-\infty}$ (*)	821.9	-	646.3	-
		Tmax (h)	0.5	-	0.5	-
		Cmax (ng/ml)	170.0	-	130.0	-

* (ng or ng equivalents x hr/ml)

Pharmacokinetics of FR900506 (FK506) in rats during repeated dosing. (see ref. 6) During 2 weeks of daily oral dosing with FK506, $AUCs_{0-24}$ were calculated on days 1, 7 and 14; an enzyme immunoassay was used to measure FK506 in plasma. No accumulation was noted at the doses of 1 and 3.2 mg/kg; the AUCs were, respectively, 8.65 and 42.08 ng x hr/ml on day 1 and 2.65 and 43.6 ng x hr/ml on day 14. However, accumulation was marked at 10 mg/kg; AUCs on days 1 and 14 were 187.3 and 2847 ng x hr/ml, a 15-fold increase. By comparing values on day 1 of this study with a previous single dose study, the AUC values at 1 mg/kg were seen to be lower than would be proportional to the AUCs at 3.2 and 10 mg/kg. (Dosing i.m. was also conducted; AUCs following a dose of 1 mg/kg showed FK506 to be approximately 6X more bioavailable by the i.m. route than by the oral route.)

Absorption: (see refs. 7-8) Using the *in situ* loop technique, the site for gastrointestinal absorption in the rat was primarily identified as the jejunum, with some absorption also occurring in the duodenum, ileum, colon, and stomach in descending order of amount absorbed (ref. 7). Oral absorption following gavage of FK506 was impaired by feeding; the AUC_{0-24} measured in whole blood of fed rats given 1 mg/kg was 16 versus 33 ng x hr/ml when the animals were fasted.

The oral dosing form of FK506. (ref 9) Hydroxypropylmethyl cellulose and an oily ethanol formula were used for formulating FK506 for use in a cardiac allograft model of rejection in rats. The latter formulation had inferior light and thermal stability and was discontinued.

Distribution: (see refs. 10-11) FK506 was shown to be 99% bound in rat, beagle dog, human, and cynomolgus monkey plasma in the range of 5-50 ng/ml (ref. 10). FK506 is taken up to a significant degree in blood cellular fractions as seen by comparing the AUCs in whole blood and plasma in the the table above. The ratio of FK506 in whole blood/plasma varied with dose; at dose of 1 mg/kg, the ratio was approximately 4:1, while at a dose of 10 mg/kg, the ratio was almost 1:1. This may indicate that at higher doses, the sites for FK506 binding in blood cells saturate, or that sites of binding in tissue saturate, resulting in a smaller volume of distribution and higher plasma concentrations. The whole blood levels were (approximately) linearly proportional to oral dose at doses of 3.2 or 10 mg/kg; however, a dose of 1.0 mg/kg resulted in blood or plasma levels (Cmax) or exposure (AUC) of FK506 which were 2-fold lower than anticipated. As seen in ref. 11, freshly drawn blood from human, beagle dog, and cynomolgus monkey was incubated with FK506 ex vivo for 1 h at 37 degrees C. 95% of FK506 was distributed in erythrocytes and 2% was found in the plasma. This proportionality held for whole blood levels of 5-50 ng/ml; however, in rats at blood levels of 5 ng/ml, 50% of the FK506 was detected in monocytes, 35%, in erythrocytes, and 15%, in plasma. The fraction of FK506 found in the plasma of rats was concentration-dependent, increasing 3 and 5 times that at 5 ng/ml when blood levels were increased 10 and 100 fold, respectively.

Tissue Distribution: (See refs. 12-14) A single dose of 1 mg/kg ^{14}C-FK506 was given i.v. or orally to rats, and sequential sacrifices were conducted at 0.5, 8, 24 and 72 h post dose. Organ levels showing greater than 1% of the dose are listed. Following oral dosing, radioactivity was noted in the stomach, small and large intestine out to 24 h post dose. Following i.v. dosing, additional organs with greater than 1% of the dose were lungs and kidney (0.5 h only), and liver (out to 24 h). No radioactivity was detectable 72 h following oral dosing; approximately 0.5% of the initial dose was still evident in liver and large intestine 72 h following i.v. dosing. The picture was slightly different when ^{14}C-FK506 was given at levels of 1 mg/kg orally or i.v., with tissue distribution monitored by whole body autoradiography; levels were quantitated using densitometric scans. Positive autoradiograms were seen in the following tissues; hypophysis, harderian gland, tongue, salivery glands, myocardium, liver, spleen, adrenal gland, stomach, gastric and intestinal walls, urinary bladder, muscles, and eye capsule at 5 and 15 min following dosing. At 8 h following dosing, only the stomach and intestines showed radioactivity of a moderate or marked degree. No radioactivity was detectable at 48 h. Following oral dosing, stomach and intestines showed moderate-marked levels of radioactivity at all time out to 24 h; no radioactivity was noted at 48 h. In non-fasted, near-term (gestation day 18) pregnant rats given 1 mg/kg orally (ref. 14), radioactivity was detected in the fetus at 8 and 24 h post dose, mainly in the blood, brain, and liver.

Metabolism: (see refs. 15-20) Metabolites which were identified following incubation of FK506 with rat hepatic liver microsomes (by HPLC, nuclear magnetic resonance spectrometry, and mass spectrometry) were 13- and 31-O-demethylated metabolites; only the latter showed significant immunosuppressive activity in a mixed lymphocyte reaction (10-fold higher IC50 than FK506) and significantly cross-reacted with the monoclonal antibody used in the enzymatic immunoassay. By infusing FK506 into the femoral or portal vein, whole blood $AUCs_{0-2}$ were compared (ref. 21). These AUCs were 268 and 136 ng x hr/ml, showing that approximately 50% of FK506 coming into the liver was metabolized immediately; alternative terms for this effects is first-pass or presystemic elimination.

Effects on renal and hepatic p450 metabolism: (see refs. 15-20). Rats were treated with oral doses of 0.4, 2, or 10 mg/kg for 7 days; livers were evaluated for microsomal p450 enzyme content; 30% increases were seen at 0.4 and 10 mg/kg, but not at 2 mg/kg, calling into question the significance of this finding. Decreased NADPH-cytochrome c reductase activity was seen (67%), but increased cytochrome b5 activity was seen. When FK506 was incubated with microsomal enzymes derived from FK506-naive and FK506-pretreated rats, no difference in metabolite profile was noted. Treatment of rats with FK506 did not induce changes in levels of kidney cortex microsomal p450 enzymes, although cyclosporin treatment did. Antibodies to rat p450 3A were generated and incubated with rat microsomal preparations along with FK506. This enzyme is inducible by pretreatment of rats with dexamethasone or rabbits with erythromycin. Microsomes from 3A-induced rats (as well as humans) metabolized FK506 to the 13-desmethyl metabolite; production of this metabolite was inhibited 82% by coincubation with the antibody directed against the 3A enzymes in the rat liver microsomal test. While the major metabolite of FK506 generated by rat and human liver microsomes has not been identified with certaintly, the major metabolite in each of these two species appeared to be identical as shown by coelution on HPLC. When microsomes from phenobarbital treated rats were incubated with FK506, 4 metabolites were identified as the 13-, 15-, or 31-mono-0-demethylated metabolites and a metabolite which was hydroxylated at the 12-position.

Effect of 23 drugs of metabolism of FK506 by human liver microsomes. (see ref. 21)Studies of the inhibition of FK506 metabolism by human liver microsomes was conducted by coincubation at 10 or 100 uM of each of the following drugs with microsomes and FK506 at 10 uM: amphotericin b*, diltiazem*, erythromycin*, fluconazole*, nilvadipine*, prednisolone*, rifampicin*, cefixime, loxoprofen, ceproflexacin, enoxacin, fosfomycin, kanamycin, lincomycin, minocycline, oflaxacin, norethindrone, ethinyl estradiol, acyclovir, cefotaxime, and phenobarbital, as well as cyclosporin A and nifedipine. The latter 2 showed the strongest inhibition of FK506 metabolism, along with the other compounds marked with a "*", consistent with the hypothesis that cytochrome P450 3A is a crucial step in metabolism of FK506. The maximum inhibition seen at 100 uM was approximately 60%.

Enterohepatic circulation of radioactivity in rat after p.o. adminstration of [^{14}C]FK506. (see ref 22) A single oral dose of 1 mg/kg of [^{14}C]FK506 (15.5 uCi/kg)was adminstered to male Sprague-Dawley rats. During the 24 h following dosing, a bile sample was collected, then injected into the duodenum of another male rat. Excretion was monitored. The conclusion of this study was limited to observing that the biliary radioactivity derived from the first rat (supposedly biliary metabolites) appeared in the feces of the second rat, not the urine.

Excretion: (see refs. 23-24) Excretion of ^{14}C-FK506 following oral or i.v. administration was seen mainly in the bile and feces; 95% of the radioactivity was found in the feces, 5% was found in the urine, and essentially none was found in expired air (see ref. 20). Delivery of ^{14}C-FK506 to nursing pups in the milk of lactating dams (see Segment III reproductive toxicity study) was evaluated (see ref 14). At 8 h following an oral dose of 1 mg/kg, radioactivity was detected in milk at a level similar to the level seen in plasma at that time (15 ng equiv./ml). In another study, Sprague-Dawley male rats were given a single or 28 daily doses of [^{14}C]FK506, and urinary and fecal levels were monitored for 24 (urine) or 48 (feces) h. Liquid chromatography (LC) profiles showed that in urine, peak U1 and U5 predominated after a single dose, but following repeated dosing, peaks U1 and U2 predominated. In feces, peaks F1 and F2 codominated the LC profiles followir a single dose, but following repeated doses, peak F2 predominated. Peak F2 and F1 migrated at the same times as peaks U2 and U1, respectively. No mass spectrometry was performed on these peaks to identify their structures.

Pharmacokinetics of FK506 in the baboon. (see ref. 25) Following an overnight fast during week 1, 3 male baboons were administered a single dose of FK506 at 1 mg/kg i.v.; during week 2, the same baboons were fasted and given 10 mg/kg orally. Levels of FK506 in whole blood and plasma were measured over a period of 24 h. Model independent pharmacokinetic parameters were calculated for both FK506 (evaluated by enzyme immunoassay) and radioactivity (evaluated by scintillation counting), and are reported in the following table.

Pharmacokinetic parameters calculated from an i.v. or oral single dose in baboons.

Route	Dose (mg/kg)	Parameters	Whole Blood FK506	Whole Blood Radioactivity	Plasma FK506	Plasma Radioactivity
I.V.	1.0	t½ (h)	12.3	13.6	9.6	28.0
		AUC $_{0-\infty}$ (*)	3956	3342	278	1307
		Vd (l/kg)	3.93	3.88	12.5	22.0
		Clearance (l/h x kg)	0.27	0.23	3.65	0.78
P.O.	10.0	t½ (h)	10.4	-	21.4	-
		AUC $_{0-\infty}$ (*)	2007	-	63	-
		Tmax (h)	2.0	-	1.7	-
		Cmax (ng/ml)	190	-	13	-
		Bioavailability	5.1%	-	2.3%	-

* (ng or ng equivalents x hr/ml)

FK506 in the plasma was rapidly metabolized and broadly distributed following i.v. dosing as shown by the relatively high clearance and large volume of distribution (Vd). FK506 was mainly distributed in the blood cellular compartment as shown by the high ratio of AUCs in whole blood and plasma. Following oral dosing, the Cmax for FK506 was 15X higher in the blood than in plasma; the bioavailability from the oral route was 5% as measured in whole blood; this value is 1/2 that in rodents.

Pharmacokinetics of plasma FK506 in baboons during repeated dosing. (see ref 26) Baboons (4 males/group) were given oral doses of 1, 3, or 10 mg/kg. Two further groups (3 males/group) were given i.m. FK506 at doses of 0.1 or 1 mg/kg. FK506 was administered daily for 28 days; pharmacokinetics parameters for FK506 were determined on days 1, 14, and 28 by immunoassay of plasma. Oral: On the basis of AUC_{0-24h}, accumulation (2-3 fold) occurred at the upper two doses (from 12 to 24 ng x hr/ml; and from 34 to 96 ng x hr/ml, respectively). AUCs at the beginning and end of the study were approximately proportional to nominal dose. The Cmax doubled with time in the upper two dose groups and was proportinal to dose by the end of the study. Intramuscular: On the basis of AUC_{0-24h}, accumulation (12-27 fold) occurred in both dose groups (from 1.6 to 19 ng x hr/ml; and from 6.5 to 174.6 ng x hr/ml, respectively). Exposures were in proportion to doses by day 14. Accumulation was marked even after the 14 day point. The Cmax also reflected accumulation (5-10 fold) of drug between days 1 and 14; little further accumulation was noted, unlike the case for AUCs. Values for Cmax increased from 0.18 to 1.1 ng/ml and from 0.6 to 8.4 ng/ml for the low and high dose groups, respectively. Tmax was

unchanged by dose or by dose period; the Tmax for i.m. dosing was approximately 5 h; the Tmax for oral dosing was approximately 1.5 h.
Comment: Note these values reflect plasma levels, not the more useful whole blood levels.

The distribution and excretion of FK506 in baboons after single i.v. doses of 1 and 10 mg/kg. (see ref 27) ^{14}C-FK506 (10 uCi/mg) was administered i..v. to a male baboon at 1 mg/kg; radioactivity in excreta was monitored for 6 days. 82% of the radioactivity was excreted in the feces and 3% in the urine; when this animal was sacrificed at day 6, no detectable radioactivity was present in tissues (similar results were obtained following similar dosing in a female). The same dose was administered to another male baboon; this animal was sacrificed and levels of radioactivity in the various organs was 37% in muscle, 24% in bile, 5% in liver, 4% in bone marrow, and 3% in each of the small intestine and fat. The whole blood/plasma ratio was 1.8. In another animal sacrificed at 24 h, the levels were 42% in large intestinal contents, 9% in muscle, and large intestine walls (7%). An oral dose of 10 mg/kg was administered to a female; one hour later, 33% of the radioactivity was found in the gastrointestinal contents, 27% in the stomach and intestinal walls, and 1.4% in the bile. At 24 h, 45% was found in the gastrointestinal contents and 11% in the walls. At 6 days, no detectable radioactivity was found in the body; 96% of the dose had been excreted.
Comment: Biliary excretion was evident in the case of i.v. administration, but not with oral administration.

Absorption, distribution and excretion studies of FK506 in baboons. (see ref. 28) ^{14}C-FK506 was administered at doses of either 1 mg/kg i.v. or 10 mg/kg orally to male and female baboons. Pharmacokinetic variables were calculated. Following a dose of 1 mg/kg i.v., the t1/2 was 16.8 +/- 3.6 and the AUC 0-infinity was 4945 +/-1034. These variables could not be calculated following the oral dose due to the low levels seen in whole blood. The AUC was approximately half of that following the i.v. dose. These values are similar to those seen in PK1.

NONCLINICAL PHARMACOLOGY STUDIES

Summary:

1. Fujii Y. Fujii S, Kaneko T. Effect of a novel immunosuppressive agent, FK506, on mitogen-induced inositol phospholipid degradation in rat thymocytes.
2. Nicoletti F, Meroni PL, Barcellini W, et al. FK506 prevents diabetes in diabetes prone BB/Wor rats.
3. Murase N, Lieberman I, Nalesnik M, et al. Prevention of spontaneous diabetes in BB rats with FK506.
4. Kawashima H, Fujino Y, Mochizuki M. Antigen-specific suppressor cells induced by FK506 in experimental autoimmune uveoretinitis in the rat.
5. Deguchi K, Takeuchi H, Miki H, et al. Effects of FK506 on acute experimental allergic encephalomyelitis.
6. Miyahara H, Hotokebucji T, Arita C, et al. Comparative studies of the effects of FK506 and cyclosporin A on passively transferred collagen-induced arthritis in rats.
7. Fujitsu T, Mori J, Ono T, Shibayama F. General pharmacology of FK506. Fujisawa Pharmaceutical Company Report CRR880186, 1988.

Review:

1. Effect of a novel immunosuppressive agent, FK506, on mitogen-induced inositol phospholipid degradation in rat thymocytes. Concanavalin A (Con A; 2 ug/ml) stimulates growth in a primary culture of thymocytes from 8-wk old Wistar rats. FK506 was seen to inhibit this growth by 80% at concentrations of 10^{-7} M. Evaluation of inositol phospholipids showed that, while Con A stimulates breakdown of phosphatidyl inositol bisphosphate, this biochemical step was not affected by coincubation with FK506, indicating the inhibitory effect of FK506 is "downstream" of cytoplasmic second messenger effects.
2. FK506 prevents diabetes in diabetes prone BB/Wor rats. Daily (6/week) i.m. injection of 0.125 mg/kg FK506 in BB/Wor (diabetes-prone) rats from the age of 27-120 days of age prevented the

hyperglycemia (7.8 rather than 33 mM/l) normally seen in this strain of rats. This model may be relevent to an autoimmune basis for insulin-dependent diabetes mellitus.

3. Prevention of spontaneous diabetes in BB rats with FK506. In a manner similar to above, oral FK506 (1 or 2 mg/kg) was administered at days 30-120 of age in BB rats. While 10% of the animals treated with 1 mg/kg started to show increased blood glucose by day 110, all animals in the 2 mg/kg groups showed normal blood glucose levels at day 120; 9/20 animals were still in the normal range at day 165.

4. Antigen-specific suppressor cells induced by FK506 in experimental autoimmune uveoretinitis in the rat. Treatment of rats with soluble retinal antigen (S-antigen) induced an autoimmune reaction, characterized as uveoretinitis (EAU). FK506 (1 mg/kg, days 0-14 following S-antigen), blocked EAU development; this response was correlated with the development of S-antigen-specific T-suppressor cells. When a second type of retinal antigen was injected on day 30 in treated rats, EAU developed subsequently.

5. Effects of FK506 on acute experimental allergic encephalomyelitis. Acute experimental allergic encephalomyelitis is used as a possible model for multiple sclerosis and is induced by injection into the footpad of an emulsion of Freund's adjuvant and homogenized guinea pig spinal cord; hind-leg paralysis and sometimes tetriplegia results. Oral doses of FK506 (1-10 mg/kg) were administered days 0-12 following sensitization; onset of EAE was delayed from 12 to 21 days (10 mg/kg).

6. Comparative studies of the effects of FK506 and cyclosporin A on passively transferred collagen-induced arthritis in rats. In this rat model of arthritis, FK506 was administered (10 mg/kg s.c.) the same day as sensitization using an injection of adjuvant and bovine collagen (CII). In controls, an arthritic condition is evident by 11 days, and antibodies to CII are detectable in the serum. Antibody production was nearly completely suppressed on day 21 in FK506-treated rats; skin testing showed suppressed reactivity until at least day 28. Subsequent rechallenge with CII resulted in acute arthritis in only 5/29 rats, showing immune responses to CII were specifically suppressed.

7. General pharmacology of FK506. In this technical report, FK506 (32 mg/kg p.o., 0.32 mg/kg i.v.) was administered to animals in a variety of acute pharmacological activity screens or at a concentration of ng/ml in isolated organ systems. No effects were seen on the following parameters: behavior of rats, locomotor activity or hexobarbital anesthesia in mice; (i.v. in anesthetized dogs) blood pressure, heart rate, electrocardiogram, or respiration rate; contractile force or heart rate in isolated guinea pig atria; acetylcholine-induced hypotensive or adrenalin-induced hypertensive responses in rats; acetylcholine- and histamine-induced contractions of isolated guinea pig ileum, noradrenalin-induced contraction of isolated rat vas deferens, resting tonus of isolated guinea pig trachea, or electrically induced-contraction of isolated rat phrenic nerve-diaphragm preparation; charcoal meal transit time or intestinal fluid accumulation in rats or spontaneous movement of isolated rabbit ileum; bleeding time in mice, ADP- and collagen-induced aggregation of rabbit platelets, spontaneous movement of isolated non-pregnant rat uterus and carrageenin paw edema in rats. The following pharmacological responses were seen: a 0.5 degree reduction in temperature in rabbits; a suppression of acetic acid-induced writhing in mice; a decrease in blood pressure (11%) and heart rate (22%) 4 h post-oral dosing (only) in rats; and an increased urine volume and sodium excretion (39%) in rats.

NONCLINICAL THERAPEUTICS

Summary, rodents: (Review follows in table form)

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2. Inagaki K, Fukuda Y, Sumimoto K, et al. Effects of FK506 and 15-deoxyspergualin in rat orthotopic liver transplantation. Transplant Proc 1989; 21: 1069-1071.

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19. Nakajima K, Sakamoto K, Ochiai T, Asano T, Isono K. Effects of 15-deoxyspergualin and FK506 on the histology and survival of hamster-to-rat cardiac xenotransplantation. Transplant Proc 1989; 21: 546-8.
20. Nakajima K, Sakamoto K, Ochiai T, Nagata M, Asano T, Isono K. Prolongation of cardiac xenograft survival in rats treated with 15-deoxyspergualin alone and in combination with FK506. Transplantation 1988; 45: 1146-8.
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SUMMARY OF NONCLINICAL MODELS OF THERAPEUTIC EFFICACY

Ref #	Species	Donor Strain	Recip Strain	Dose, mg/kg x days*	Route	Comments (incidence; days of mean graft survival or mean survival time; model; results or qualifiers)
1	Rat	ACI	LEW	.1 x 14	IM	5/5, > 100 days; liver orthograft
2	Rat	PVG	LEW	1 x 14	SC	5/9, 33-48 days; 4/9, > 70 days; liver orthograft
3	Rat	DA	AUG	1 x 14	PO	5/9, 9-11 days; 4/9, > 41 days; liver orthograft
4 & 5	Rat	LEW	-	.3 x ? 1 x 2	IV	3/10 > 10 days; 8/10 > 10 days; alleviated hepatic ischemia
6	Rat Mouse	F344 C57BL6 (mouse)	WKA WKA	3.2 x 10 .3 x 10 3.2 x 10	IM IM IM	8/8, 39-47 days; skin allograft 10/10, > 100 days; cardiac heterograft 8/8, 40-64 days, skin xenograft 7/7, 2-3 days, cardiac xenograft
7	Rat	ACI	LEW	10 x 14	IM	5/5, 13-27; presensitized cardiac heterograft
8	Rat	Balb/c (mouse)	LEW	3 x 14	PO	20% graft survival at 14 days; mouse neonate to rat xenograft
9	Rat	F344 ACI	WKA F344	.32 x 10 1 x 10	IM IM	1/11, 2 days; 10/11, > 100 days 2/10, 48-66 days; 8/10, > 100 days; cardiac heterograft
10	Rat	WFu	BUF	.1 x 3 3 x 3	IM, FK506 PO, Cy	Sub efficacious doses of FK506 and Cyclosporin A ↑MST to 79 days from 35 or 7, respectively; cardiac heterograft
11	Rat	ACI	LEW	 1.3 x ... 1.3 x ... 1.3 x ... 1.3 x ... 1.3 x ...	IM	cardiac orthograft: 1) ...14; days 0-13; MST 88 days; 2)....3; days 4, 5, and 6; MST 91 days 3)....3; days 5, 6, and 7; MST 50 days heterotopic liver: 4)....3; days 4, 5, and 6; MST > 100 days 5)....3; days 6, 7, and 8; MST 11 days
12	Rat	-	LEW	-	-	LEW rats from study #11: long term survivors in group 4) were rechallenged with: ACI heart regraft; MST 45 days; ACI skin or BN heart, MST 10-14 days.

SUMMARY OF NONCLINICAL MODELS OF THERAPEUTIC EFFICACY						
Ref #	Species	Donor Strain	Recip Strain	Dose, mg/kg x days*	Route	Comments (incidence; days of mean graft survival or mean survival time; model; results or qualifiers)
13	Rat	BN	LEW	1.3 x ... 1.3 x ... 1.3 x ...	IM	...14; days 0-13; MST 87 days ....2; days -2 and -1; MST 30 days ....4, days -4 to -1; MST 32 days cardiac heterograft
14	Rat Dog	ACI Mongrel	LEW Beagle	1.3 x 14 1.5 x 90	PO PO	MST 87 days; cardiac heterograft MST 50 days; kidney orthograft
15	Rat	BUF	LEW	1 X 3 (-1 - 1) 1 x 13	IM IM	W/O Bone marrow, MST 8 days W/, MST 24 days W/O, MST 44 days W/, MST > 100 days cardiac heterograft W/WO BUF bone marrow, injected on day 0 @ 1 x 10^8 cells i.v.; also MLR suppressed by CD4+ cells from tolerant rats.
16	Rat	ACI	LEW	1.3 x 14 (-3 - 10 days)	IM	control; MST .27 days FK506; 2 days presensitized by prior skin graft; cardiac heterograft model of hyperacute rejection
17	Rat	DA	PVG	1 x 13	IP IM	MST for both routes = 26 days IM more consistent; 3-fold longer median ST; cardiac heterograft
18	Rat	F344 C3H/He (mouse)	WKA WKA	.32 x 10 3.2 x 10	IM IM	MST > 50 days; cardiac heterograft MST 2 days; ineffective MLR on human peripheral LYM, IC_{50} = 1 nM.
19	Rat	SPF Hamster	F344	.5 x 3	IM	hamster/rat cardiac xenograft FK506 ineffective; 2 day survival
20	Rat	Golden Hamster	F344	.5/day + DSG 3/day	IM IM	FK506 alone, ineffective; Deoxyspergualin (DSG) alone, MST 3; DSG + FK506, MST 14. Synergism evident; Hamster to rat cardiac xenograft
21	Rat	F344	LEW	.32 x 14	IM	2/10, MST 53-70; 8/10, MST > 150 days; small intestine heterograft
22 & 24	Rat	F1 LEW ACI	LEW F1 LEW	2 (1- 6); then 1 (6-28)	IM	MST 83 days; HVG model MST 188 days; GVH model MST 51 days; HVG and GVH (F1 = offspring of the ACI x LEW); small intestine heterograft.
23	Rat	BN	LEW	2 x 4	IM	8/9 MST >180 days, small intestine orthograft
25	Rat	F344	WKA	.3 x 10	IM	MST > 180 days; lung orthograft
26	Rat	WKAH	F344	.5 (1-7) .3 (7-14); .1 (14-)	IM	FK506 efficacious; pancreatico-duodenal heterografts
27	Rat	ACI	LEW STZDM	1 x 4	IM	MST 32 days; pancreatic heterograft into streptozotocin/diabetic rats

SUMMARY OF NONCLINICAL MODELS OF THERAPEUTIC EFFICACY

Ref #	Species	Donor Strain	Recip Strain	Dose, mg/kg x days*	Route	Comments (incidence; days of mean graft survival or mean survival time; model; results or qualifiers)
28	Rat	BN	LEW	.64 x 14	IM	MST >70 d[illegible]; small intestine heterograft
29	Rat	WKA	LEW STZDM	3.2 x 3	SC	MST >42 days, islet heterograft to liver
30	Rat	WKA	LEW STZDM	1 x 7	IM	MST > 54 days; islet heterograft to liver
31	Rat	WKA	LEW STZDM	.32 x 7	SC	MST > 45 days; islet heterograft to kidney capsule
32	Rat	WKA	LEW STZDM	5 mg	SC	continuous infusion; MST > 61 days; day 7 plasma level 1.6 ± .3 ng/ml
33	Rat	WIS LEW	ACI STZDM	1 (1-14) then 1, once weekly	IM	WIS, MST > 171 days; WIS presensitized, MST > 128; WIS + LEW, MST > 99 days; islet heterograft to kidney capsule
34	Rat	LEW	WIS STZDM	.2 x 14	IPo.	intraportal cannulation, continuous infusion; MST 49 days; islet heterograft to liver via intraportal injection
35	Rat	ACI	LEW TBI	1.5 (3-16); 1 (3-9) .1 (10-)	IM IM	10% with GVHD, MST >60; 0% with GVHD, MST >60; TBI = Total body irradiated recipients
36	Rat	F344 ACI WKA LEW C57BL6	WKA WKA F344 F344 WKA	3.2 x 10	IM	MST 43 days; skin allograft MST 63 days MST 53 days MST 126 days MST 55 days; skin xenograft
37	Rat	BN	WAG	.5 /day	IM	MST 26 days; skin allograft
38	Rat	LEW	PVG	.64 x 14	IM	MST 50 days; vascularized limb allograft
39	Rat	BN	F344	5 x 14	IM	MST 102 days; vascularized limb allograft
40	Rat	BN	F344	10 x 1 then 1.5, once/wk	IM	MST >300 days; limb allograft
41	Rat	LEW	PVG	.64 x 14	IM	MST 50 days; 4/12 long term survivors; CTL assay, specific cytotoxicity 50% lower in tolerant animals than in rejectors; MLR assay, 20% lower in tolerant animals that in rejectors.

*day 0 = day of engraftment

FK506 was effective (dosing typically for 14 days from engraftment with 1 mg/kg, i.m.) in a number of different animal models (cardiac, liver, small intestine, islets, lung, or skin) of heterotopic and orthotopic allograft rejection. Treatment often resulted in graft maintenance for greater than 70 days, or more than 10-fold increases in graft survival. More limited efficacy was noted in presensitized or xenograft models (mouse or hamster to rat) of cardiac and skin graft rejection; these represent models of hyperacute rejection as antibodies to the foreign tissue are already

circulating in the donor prior to engraftment. In these cases, higher doses (3.2 to 10 mg/kg) were needed to achieve slight to modest increases (2-10 fold) in mean survival times. Combinations of "subefficacious" (as seen in monotherapy) doses of cyclosporine A or deoxyspergualin showed synergistic effects with FK506 in cardiac xenograft and allograft models. In a comparative model of GVH and HVG rejection, treatment with FK506 (1-2 mg/kg for 28 days) was seen to be twice as effective in inhibiting GVH rejection. Interestingly, FK506 was also effective in several models of vascularized limb allografts; in one case, a treatment course of a single high dose (10 mg/kg), followed by once/weekly maintenance doses (1.5 mg/kg) resulted in apparently normal-gaited, long-term (>300 days) survivors. In a case where timing of FK506 treatment was evaluated relative to cardiac or liver engraftment (day 1), a critical window of immunosuppression was identified; drug exposure had to start before day 5 in order to achieve 10-fold increased in MST. In cases where in vitro assays were performed using lymphocytes from engrafted animals, the cytotoxic T lymphocyte (CTL) assay correlated better with graft acceptance than did the mixed lymphocyte response assay (MLR).

Summary (Primates):

1) Imventerza, O. et al. Renal transplantation in baboons under FK506. Transplant Proc 1990 22:64-5.
2) Monden M. et al. A potent immunosuppressive effect of FK506 in orthotopic liver transplantation in primates. Transplant Proc 1990 22: 66-71.
3) Ochiai, T. et al. Studies on FK506 in experimental organ transplantation. Trnasplant Proc 1988 20:209-14.
4) Todo, S. et al. Immunosuppression of canine, monkey, and baboon allografts by FK506: with special reference to synergism with other drugs and tolerance induction. Surgery 1988 104:239-49.
5) Collier S., et al. FK506 in experimental renal allografts. Transplant Proc 1987 19:3975-7.
6) Collier D., et al. FK506 in experimental renal allografts in dogs and primates. Transplant Proc 1988 20: 226-8.
7) Calne R. Observations about FK506 in primates. Transplant Proc 1987 19:63.
8) Todo S., et al. Renal transplantation in baboons under FK506. Surgery 1989 106:444-51.
9) Flavin T. et al. Initial experience with FK506 as an immunosuppressant for nonhuman primate recipients of cardiac allografts. Transplant Proc 1991 23:509-10.
10) Hildebrandt A., et al. FK506: short- and long-term treatment after cardiac transplantation in nonhuman primates. Transplant Proc 1991 23:509-10.
11) Ericzon BG, et al. Pancreaticoduodenal allotransplantation with FK506 in the cynomolgus monkey. Transplant Proc 1990 22:72-3.
12) Ericzon B-G., et al. Effect of FK506 on glucose metabolism in the cynomolgus monkey: studies in panoreatic transplant recipients and nontransplanted animals. Transplant Proc 1991 23:511.

Review (Primates):

1) Renal transplantation in baboons under FK506. Baboon were given 12-18 mg/kg FK506 orally for 90 days following renal orthografts. Half of the groups survived more than 90 days, but evaluation of plasma levels were unable to distinguish a difference in dose levels which could account for survival results. The relatively high doses which demonstrated efficacy (relative to dog or rat models) prompted an investigation of the direct lymphocyte sensitivity as monitored in vitro by a mixed lymphocyte assay. Results of this assay revealed that baboon lymphocytes are 10 X less sensitive to the proliferative inhibition caused by FK506, as compared to rat, dog, and human lymphocyte responses.

2) A potent immunosuppressive effect of FK506 in orthotopic liver transplantation in primates. Liver orthografts were performed in cynomolgus monkeys, who were subsequently given i.m. FK506 at 1 mg/kg for several days, followed by approximately 10 mg/kg/day orally. Plasma levels were monitored, and doses were adjusted to maintain plasma levels of 1-2 ng/ml. Of the initial 15 monkey engrafted, 12 survived past day 3: of those, 4 were excluded due to operative

complications; 4 were used as controls, and 4 were given FK506. The 4 animals used as controls died by 6, 8, 12, and 63 days. Of the 4 treated animals, 1 died at day 39 of sepsis (possibly due to bile leakage), and the remaining 3 monkeys lived to be withdrawn from FK506 at days 104, 105, and 132. These animals were still alive at day 336. Tolerance was evidently induced in these monkeys.

3) Studies on FK506 in experimental organ transplantation. Female beagles were given renal orthografts and treatments with 0.08 - 0.32 mg/kg i.m. FK506 or 0.32 - 1.0 mg/kg oral FK506. A dose of 0.16 mg/kg i.m. or 1.0 mg/kg oral was effective, but was associated with intussusception and death in 8 animals, and anorexia. No histopathological evidence of ulcers was evident on necropsy. Median survival time was >165 days for the 11 animals receiving the optimal doses.

4) Immunosuppression of canine, monkey, and baboon allografts by FK506: with special reference to synergism with other drugs and tolerance induction. In a study of short courses of FK506 to beagles which were orthografted with a mongrel kidney, comparison of 3 days of i.m. FK506 at a dose of 1 mg/kg was made. Groups of 5 each were given FK506 on day 1-3, 4-6, or 7-9. The treatment was associated with prolonged survival in all groups, but was most effective on days 4-6. Two cynomolgus monkeys were given FK506 at oral doses of 0.3 or 1.0 mg/kg following renal transplantation; these animals lived to 95 days (rather than 9 in controls); FK506 plasma levels were 0.4 - 3.0 ng/ml. In tests of combination therapies, beagles receiving 0.5 mg/kg of FK506 oraly in combination with 5 mg/kg of either cyclosporin A or prednisone were evaluated following renal (mongrel) orthografts. Response to FK506, cyclosporin, and prednisone was excellent, but the omission of one drug or reduction of doses reduced the efficacy of the treatment.

5) FK506 in experimental renal allografts Mongrel dogs were given FK506 at 1 mg/kg i.m. (dosage was adjusted downwards by 1/2 every 84 days); grafts in this group survived 115-250 days. Baboons received orthografted kidneys (histocompatibility matched by MLR); FK506 was given in group 1) at 1 mg/kg i.m. for 30 days, then 0.2 mg/kg (30 days), and 0.1 mg/kg (30 days); 2) 1 mg/kg i.m. followed by 0.1 mg/kg; and 3) 0.05 mg/k i.m. daily. Widespread vasculitis was noted in dogs; hyperglycemia was noted in baboons at all doses above 0.05 mg/kg. Only one baboon (group 1) maintained a functional graft longer than 36 days (control graft survival was 10 days).

6) FK506 in experimental renal allografts in dogs and primates. Renal orthografts were performed in mongrel dogs and baboons; baboons were dosed with FK506 at 1 mg/kg orally for 28 days; dogs were given 0.5 or 1 mg/kg orally. [In a separate study, mongels (2) were given 2 mg/kg/day for 28 days and sacrificed for histopathological assessments of toxicity. Vasculitis, elevated ALT and AP levels were noted, and the dogs lost weight.] Following engraftment, myocardial necrosis secondary to vasculitis, intussusception, hepatic failure, and rejection (in 6/12 animals) were noted at doses which were effective (greater than 0.5 mg/kg orally). Thus toxicity seem to preclude using the dog as a model for graft rejection and immunosuppression using FK506.

7) Observations about FK506 in primates. See number 5; repeated report of the same data.

8) Renal transplantation in baboons under FK506. Baboons were given renal orthografts and subsequently were treated with FK506 at 2, 6, 12, or 18 mg/kg for 90 days. Best graft survival was obtained at the two highest doses; while plasma levels of 3-5 ng/ml were detected in the groups receiving the top 3 doses. Therefore, plasma levels were not predictive of graft survival. Mixed lymphocyte response assays (MLR) did not correlate with outcome. 1/5 baboon treated with 12 or 18 mg/kg showed long term (>200 days) graft acceptance after treatment was discontinued.

9) Initial experience with FK506 as an immunosuppressant for nonhuman primate recipients of cardiac allografts. Male cynomolgus monkeys were given i.m. FK506 (for 5 days at 1 mg/kg, and then at 10 mg/kg orally, reduced to 5 mg/kg every other day) following a cardiac heterograft. This dosing protocol cause no obvious toxicities, and was associated with prolonged graft survival in several animals (healthy grafts at sacrifice at days 63 and 75). Histopathological evaluation of graft tissues revealed lymphocytic infiltrates consistent with mild rejection, but no coronary disease impairing beating.

10) FK506: short- and long-term treatment after cardiac transplantation in nonhuman primates. Chacma baboons (14-35 kg) were given cardiac heterografts and treated with FK506 at 2 mg/kg i.m. for 14 days, 0.5 mg/kg i.v. for 14 days, or 1 mg/kg i.m. for 42 days. The most effective treatment was the first, with a mean graft survival of 97 days (n=5). Other signs of immunosuppression were reactivated tuberculosis, *Pseudomonasl pneumonia, Klebseilla septicaemia, and camphylobacter enteritis.* Uremia and renal failure were side effects of FK506 treatment, seen in the third group.

11) Pancreaticoduodenal allotransplantation with FK506 in the cynomolgus monkey. Cynomolgus monkeys were given i.m. FK506 at 1 mg/kg for 4 days, followed by 10 mg/kg orally, subsequent to pancreatoduodenal orthografting. In general, FK506 was well tolerated; normoglycemia was evident 30-70 days post-transplant. Only 1/3 animals required a 50% dose reduction to ameliorate an increase in serum creatinine and weight loss.

12) Effect of FK506 on glucose metabolism in the cynomolgus monkey: studies in pancreatic transplant recipients and nontransplanted animals. Glucose tolerance was evaluated in cynomolgus monkeys and monkeys receiving pancreatic orthografts. FK506 treatment was administered at 1 mg/kg orally or i.m. in normal animals; following transplant, FK506 was given at 1mg/kg i.m. for 4 days, followed by 5-10 mg/kg orally. In normal monkeys, glucose tolerance was impaired; in transplanted monkeys, blood glucose levels were normal up to 35 days following transplant, but glucose intolerance was evident at 90 days. Glucose intolerance did not correlate with the degree of rejection seen in the graft tissue, so this response probably reflected drug toxicity. Glucose tolerance was improved by a dose reduction.

NDA SUMMARY

Toxicity in rats: As seen in the table below, the rat organs and systems which showed adverse reactions to oral treatment with FK506 are listed in order of most to least sensitive; sensitivity was indicated by the lower doses and shorter times associated with effects. While this is not a comprehensive list of all changes in the organs listed (see tables in the section labelled "toxicity studies - reviews"), the effects selected for presentation in this table were, for a given organ, the most consistently seen among the different safety studies performed. The order of sensitivity is immune system > kidney > pancreas > liver > blood > reproductive organs > nerves. These results may misrepresent the sensitivity of the pancreas to injury by FK506. In a special toxicity study (results not included in this table), glucose intolerance which was unaccompanied by increased fasting blood glucose levels, was noted following 2 weeks of dosing at 1 mg/kg. These data were reported in a non-GLP study; if born out by further reseach, it would indicate the pancreatic islets as the most sensitive target for FK506 toxicity. Lens opacity or lenticular degeneration was noted in several toxicity studies in rats and is likely to result from adverse changes in glucose metabolism; these effects are also well-known in diabetic humans. For this reason, lenticular changes are included in the table as a marker of pancreatic toxicity.

For most of the effects noted, a clear (inverse) relationship exists between the time the effect appears and the dose causing the effect, particularly in the cases of decreased WBC and increased serum lipids. For these effects, increasing dosing periods (4 to 26 weeks) decreased the dose needed by 6-fold. A similar relationship is evident for weight loss, changes in erythrocytic parameters or glucose, and decreased serum proteins; in some cases, the decrease in dose was not proportional to the increase in dosing period; in all cases, toxicities apparent at later times required less drug to elicit the effect. Neurotoxicity was evident at 52 weeks at a dose equivalent to clinical dose, evidenced by tremors and circling; this may or may not be related to signs of fecal encephalomyelitis seen in the 13 week study at a dose of 3.2 mg/kg.

SUMMARY OF TOXIC EFFECTS IN RATS: DOSES (MG/KG) AND EARLIEST TIMES EFFECTS WERE OBSERVED

Organ or System	Effect	2 Weeks	4 Weeks*	13 Weeks	26 Weeks	52 Weeks
IMMUNE	Δ Organ weights	3.2	3.2	3.2		1.5
	↓WBC or LYM		3.2	0.5♂	0.5♀	
KIDNEY	↑BUN or creatinine	3.2	3.2	0.5♀	1.5♂	
	Basophilic tubules		3.2	1.0		
PANCREAS	Δ Urine or Serum Glucose	10.0	3.2		1.5♂	
	Lens opacity			1.0♂		1.5♀
LIVER	↑Lipids		3.2	1.5♀	0.5	
	↓Serum proteins	32		1.5♂		1.5♀
BLOOD	Δ RBC or hemoglobin	32	3.2	3.2	1.5♂	
REPRODUCTIVE	Δ Organ weights		3.2♀	3.2♂		1.5♂
NERVES	Sciatic nerve damage & tremors			3.2♂		1.5
GENERAL	↓ Weight gain	3.2		3.2♂		0.5♂
	Weight loss	32				1.5♂

* performed with 21-day-old rats (weanlings)

In general, male rats exhibited a greater sensitivity to FK506 at a given dose and time compared with females, as evidenced by the absence of any significant weight loss or decreased BWG in any study for females. However, several effects appeared first in females; kidney toxicity at week 13, lipid increases at week 13, and reproductive organ weight changes at week 4. Therefore, no strong evidence exists for a sex-selective effects of FK506. However, one effect was seen almost

exclusively in males, lens opacity (also 1 male with cataract at week 52); while this effect was probably secondary to pancreatic toxicity and glucose intolerance, the ocular changes seen in preclinical studies indicate the need for regular eye exams in human patients. Myocardial inflammation was evident in one study following 4 weeks of intravenous exposure to FK506 at a dose of 1 mg/kg. This i.v. dose is equivalent to (12% bioavailable; therefore, 8.3 mg/kg oral dose) an oral dose in humans (corrected by a factor of 6.3 for body surface area conversion) of 1.3 mg/kg; 4-fold higher than the recommended therapeutic dose. Therefore, myocardial inflammation due to i.v. FK506 is not likely to present a clinical risk; clinical dosing with the i.v. form should not extend beyond 4 weeks without more intensive i.v. animal testing.

Toxicity in baboons. Toxicity studies were conducted in baboons using oral gavage to a maximum duration of 52 weeks. For dose conversions, 1 mg/kg is equivalent on a body surface area basis to a human dose of 0.4 mg/kg, approximately equal to the recommended clinical dose of 0.3 mg/kg. The acute effect of 250 mg/kg orally or 2 mg/kg i.v. was anemia. In 13-week subchronic studies, targets of FK506 toxicity were: at 3 mg/kg (4X the clinical dose), erythrocytes (slight anemia); at 6 mg/kg (8X the clinical dose), organs of immunity (lymphoid atrophy), pancreas (glucose intolerance), and kidney (decreased serum calcium and increased urea), and; at 9 mg/kg (12X the clinical dose), liver (decreased serum proteins and increased LDH). At 18 and 36 mg/kg, neurotoxicity (tremors) and hematotoxicity (decreased platelets, neutrophils, and lymphocytes) were seen. In 52 week studies, toxicities to organs of immunity, the pancreas, and the gastrointestinal mucosae (discoloration and congestion) were seen at 1 mg/kg. The latter effect was unique, being expressed only in the chronic study. In an i.v. study of 4 weeks duration, FK506 showed a toxicity profile similar to that seen in oral studies. Therefore, the order of sensitivity of organs or systems to the adverse effects of FK506 was seen to be immune system = kidney = pancreas > liver> nervous system = blood.

Toxicity in dogs (vomiting and vasculitis) at minimally immunosuppressive doses precludes the use of canine models for either efficacy or toxicity. Rabbits given i.v. FK506 showed mortality, decreased erythrocytic parameters, and kidney functional changes at 0.2 mg/kg, body weight loss and cardiovascular toxicity at 0.1 mg/kg, and decreased body weight gain and increased blood glucose at 0.05 mg/kg. No NOAEL was demonstrated in this study. The cardiovascular changes exhibited in this study were unique to the rabbit. No evidence of immunosuppression (thymic or other lymphoid tissue atrophy) was seen at any dose tested here.

Reproductive toxicity: Significant effects on the reproductive capacities of the pregnant rats were seen at 1 mg/kg, which did not cause frank maternal toxicity. This dose, which was equivalent to 0.5 times the clinical dose, caused increased pre-implantation losses. At doses 1.5 times the clinical dose, maternal (and paternal) toxicity was seen; adverse effects on maternal reproductive functions and fetuses were noted, and included parturition, pup viability, and a low incidence of malformations.

Segment 1 in rats: FK506, when given orally to rats at a dose of 1.0 mg/kg (to male and female rats prior to and during mating as well as to dams during gestation and lactation) was not associated with maternal or paternal toxicity but was associated with adverse effects on female reproduction and embryolethality; these effects occurred in rats at 1X the clinical maintenance dose. Effects on female reproductive function (parturition) and embryolethal effects were indicated by a higher rate of pre-implantation loss and increased numbers of undelivered, nonviable pups. FK506, when given at a dose of 3.2 mg/kg, was associated with maternal and paternal toxicity, as well as reproductive toxicity including marked, adverse effects on estrus cycles, parturition, pup viability, and pup malformations; these effects were evident at 3X the clinical maintenance dose. Toxicities to adult rats were indicated by neurotoxicity, reduced weight gains and food

consumption, and abnormal clinical signs in males; and reduced food consumption during gestation and lactation in females. Adverse effects on reproductive parameters included 1) increased copulatory intervals, 2) increased pre- and post-implantation loss of fetuses (resulting in smaller litter sizes), and 3) markedly decreased numbers of dams delivering. No reduction in male or female fertility was evident. Adverse effects seen in pups were markedly reduced viability and a low incidence of malformation (3 pups from 3 dams).

Segment II in rats: FK506, given orally at 3.2 mg/kg to rats, was associated with maternal toxicity (decreased food consumption and body weight gain), and caused an increase in late resorptions, decreased numbers of live births, and decreased pup weight and viability. No significant effects were seen on pup growth, developmental maturation, behavior, or reproductive function.

Segment III in rats: The Segment III study in rats evaluated doses of 0.32, 1.0, and 3.2 mg/kg, dams were dosed after organogenesis and during lactation. Maternal toxicity was evident at 3.2 mg/kg and expressed as decreased body weight gains and food consumption during gestation, decreased food consumption during lactation, and increased body weight gains during lactation. Also evident at this dose was reduced pup viability during lactation days 0-4 and reduced pup weights which were evident as late as 20 weeks of age. The only effect seen at doses below those which were maternally toxic was reduced pup weights in litters from dams given 1.0 mg/kg. These lower pup weights were evident only during the later stages of lactation, and may indicate that FK506 inhibits lactation.

Segment II in rabbits: Oral dosing with FK506 in rabbits at 0.1, 0.32 and 1.0 mg/kg during organogenesis was associated with maternal toxicity as evidenced by decreased food consumption and decreased body weight gains; these doses were equivalent to 0.03, 0.09, and 0.3 mg/kg in humans following correction by a factor of 3.3 for differences in body surface area. Adverse effects on maternal reproductive parameters seen at 0.32 and 1.0 mg/kg (equivalent to clinical doses of 0.1 and 0.3 mg/kg, or 1/3X and 1X recommended clinical doses) were abortions. Adverse effects on the fetus were seen only at 1.0 mg/kg and included increased incidences of malformations and developmental variations.

Nonclinical Efficacy and Pharmacology in rodents: FK506 was effective (dosing typically for 14 days from engraftment with 1 mg/kg, i.m.) in a number of different animal models (cardiac, liver, small intestine, islets, lung, or skin) of heterotopic and orthotopic allograft rejection. Treatment often resulted in graft maintenance for greater than 70 days, or more than 10-fold increases in graft survival. More limited efficacy was noted in presensitized or xenograft models (mouse or hamster to rat) of cardiac and skin graft rejection; these represent models of hyperacute rejection as antibodies to the foreign tissue are already circulating in the donor prior to engraftment. In these cases, higher doses (3.2 to 10 mg/kg) were needed to achieve slight to modest increases (2-10 fold) in mean survival times. Combinations of "subefficacious" (as seen in monotherapy) doses of cyclosporine A or deoxyspergualin showed synergistic effects with FK506 in cardiac xenograft and allograft models. In a comparative model of GVH and HVG rejection, treatment with FK506 (1-2 mg/kg for 28 days) was seen to be twice as effective in inhibiting GVH rejection. Interestingly, FK506 was also effective in several models of vascularized limb allografts; in one case, a treatment course of a single high dose (10 mg/kg), followed by once/weekly maintenance doses (1.5 mg/kg) resulted in apparently normal-gaited, long-time (>300 days) survivors. In a case where timing of FK506 treatment was evaluated relative to cardiac or liver engraftment (day 1), a critical window of immunosuppression was identified; drug exposure had to start before day 5 in order to achieve 10-fold increased in MST. In general, FK506 appeared to be effective in significantly prolonging allograft survivals in rats when dosing occurred for at least 2 weeks starting at engraftment; efficacy was prolonged by intermittant maintenance doses which were reduced 5-fold in frequency or magnitude. In nonclinical pharmacology assays of in vitro thymocyte proliferation and in vivo models of autoimmune disease (diabetes in BB rats, experimental uveoretinitis), FK506 was

effective (at 10 nM or 1-2 mg/kg, respectively) in inhibiting responses or significantly delaying the onset of spontaneous pathogenesis. At 10 mg/kg, FK506 was effective in delaying the onset of acute experimental allergic encephalmyelitis or preventing collagen induce arthritis. In the case of uveoretinitis, rechallenge with the same antigen elicited no response, but challenge with a second, novel antigen caused disease progression. This may indicate that FK506 specifically suppressed the development of immune reactivity (such as cytotoxic T-lymphocyte responses) to only the antigen presented during the period of drug exposure.

While dosing was largely performed i.m. in animals, a pharmacokinetic study showed that bioavailability of FK506 from the i.m. route as 6 fold higher than oral. On this basis, a dose of 1 mg/kg i.m. is approximately equivalent to an oral dose of 6 mg/kg; by surface area conversion (rat to human, dividing by 6.3), an equivalent human dose would be approximately 1 mg/kg. A five fold reduction for maintenance dosing would indicate a human maintenance dose of 0.2 mg/kg, very close to the 0.3 mg/kg currently recommended in the clinic. Therefore, by the same methods applied to toxic doses for the purpose of human risk assessment, animal doses which are efficacious appear to convert to human efficacious doses.

Nonclinical Efficacy of FK506 in baboons. The studies contained in this submission elucidated the nonclinical efficacy of FK506 and target organ toxicities in baboons, a primate which showed a low sensitivity to the immunosuppressive effects of FK506 when compared with rodents or dogs. In vitro assays of lymphocyte susceptibility to immunosuppression (MLRs) showed that baboon lymphocytes were 10-fold less sensitive to FK506 than were lymphocytes from rats, dogs, or humans. This may be important for GVH responses in baboon xenografts to human recipients. Doses which impair human HVG responses are likely to be ineffective against "carried-over" baboon lymphocytes, which may be capable of mounting a reaction to human tissue.

Nonclinical Pharmacokinetics in rats: From the results of two studies, approximate bioavailabilities of FK506 were estimated; from the oral route, FK506 was 12% bioavailable (relative to i.v.); from the i.m. route, FK506 was 6-fold more bioavailable than from the oral route, or approximately 60% bioavailable. There is little information on pharmacokinetics of FK506 following repeated, especially chronic, dosing of rats with FK506. All risk assessments will therefore be based on consevative body surface area conversions. As 1) accumulation following 2 weeks of oral dosing was noted at 10 mg/kg but not at 3.2 mg/kg, and 2) as 3.2 mg/kg was the highest dose tested in 3 month studies in adult (and 1 month study in weanling) rats; toxicities seen in these studies is not likely to be due to increasing accumulation of drug. However, as the pharmacokinetic study only evaluated levels during and following 2 weeks of dosing, this does not preclude the possibility that long term alterations in hepatic metabolism (or other systems) may occur resulting in an altered pharmacokinetic profile. In general, absorption of FK506 from the oral route was impaired by food, and occurred mainly in the small intestine. FK506 is highly bound to plasma proteins, is distributed mainly in the blood cells, intestines and the liver, and undergoes significant (50%) first pass metabolism. Metabolites were rapidly generated, were typical of Phase I liver metabolism (hydroxylated and demethylated), and were excreted mainly in the bile. Clearance of FK506 was multiphasic with a t½ of 6 h for the parent drug, or approximately 11 h for radioactive metabolites. Two metabolites have been evaluated which were found to retain significant, if lesser, immunosuppressive activity. The major metabolite generated by in vitro incubations of FK506 with human and rat liver microsomes was the same as shown by comigration on HPLC. FK506 treatment for 7 days did not appear to cause the induction or reduction of liver enzymes or the production of novel metabolites; but this observation does not rule out the possibility that chronic exposure to FK506 or pathophysiologic changes may cause alterations in rates of liver metabolism. The hepatic p450 enzyme 3A may be important in FK506 metabolism. Exposure in clinically

relevant ranges appears to be proportional to dose following single doses, but as stated above, accumulation may result from repeated doses of 10 mg/kg (equivalent to 1.6 mg/kg in humans, 5X the clinical dose). Blood distribution was altered by increasing the dose from 1-10 mg/kg: blood cell or tissue sites may saturate, causing increased proportions of FK506 to be found in plasma. While 1.6 mg/kg may be a supra-clinical dose, these observations may be cautionary for patients with liver dysfunction or failing liver grafts.

Pharmacokinetics in baboons. The only repeated dose pharmacokinetic study performed using FK506 in baboons measured plasma, rather than whole blood, FK506. The bulk of the drug is now known to be found in the blood cell fractions, mainly in erythrocytes: therefore, measure of plasma FK506 are virtually without utility in guaging animal exposures. FK506 is mainly cleared in the bile and feces, is distributed widely in the body, and is similar in these parameters for both baboons and rats. The bioavailability of oral FK506 in baboons is approximately 5%.

Vehicle toxicity: Several studies evaluated the toxicity of HCO-60, the i.v. vehicle for FK506 used clinically. HCO-60 was found in an i.v., 4-week study in rats to have a NOEL of 100 mg/kg. Doses of 1000 mg/kg showed distinct hematotoxicities, evidenced by decreases in lymphocytic, erythrocytic, thrombocytic, and myeolocytic parameters. Vacuolated macrophages were evident in many organs at this dose. HCO-60 was negative in two tests of genotoxic potential, the mouse micronucleus assay, and the chromosomal aberration test using chinese hamster lung cells. While HCO-60 is mainly associated with histamine release and anaphylaxis in dogs, a study reported here showed one unexpected death in a group of 3 guinea pigs tested. While sensitization in this model should increase chances of detecting any immediated hypersensitivity or anaphylactic responses to HCO-60, 10 further guinea pigs were given repeated injections to induce sensitization, and none died. This finding is paradoxical, but cannot be dismissed as "unrelated" to HCO-60, therefore caution is warranted in using this vehicle, related chemically to Chremophor. Clinically, up to 0.15 mg/kg of FK506 is used, resulting in a dose of 6 mg/kg of HCO-60 in humans. This dose is equivalent (on a body surface area basis, multiplying by 5.5) to a dose in the guinea pig of 33 mg/kg; so the reaction in the guinea pig occurred following a dose 5X higher than that received by patients. These cautions are already well known medically. Occasional allergic reactions in patients receiving these agents has been noted.

Carcinogenicity: The doses used in the 13-week dietary range finding study in rats were too low for use in determining the MTD and appropriate doses for a 2-yr carcinogenicity study in rats. This opinion was also held by Dr. A. Taylor, and expressed to the company in 1990. Two rat carcinogenicity studies have been done; none have been submitted to the IND; the second study is being performed with higher doses than the first, and is currently ongoing.

Antigenicity: In rat and guinea pig in vivo antigenicity models, FK506 was negative for inducing antibody responses, either in the presence or absense of adjuvant.

Product: Light damage (resulting from 30,000 lux for 14 days) may lead to toxic degradation products in the clinical i.v. formulation of FK506 (see chemist's review for product handling precautions).

CONCLUSIONS

The following conclusions can be drawn from studies conducted in rats.

A) Adverse reactions in animals followed a predictable pattern, with the severity of toxicities increasing with dose and time; no mortalities or precipitous changes occurred. Target organs for

FK506 toxicity included the immune, nervous, and reproductive systems as well as the pancreas, kidney, liver, and blood. All of these organs were adversely affected by 1 yr of treatment in rats receiving 1.5 mg/kg; based on a body surface area correction factor of 6.3 for rats, the equivalent clinical dose would be approximately 0.23 mg/kg, or approximately equal to the recommended clinical dose (0.3 mg/kg). Most organs/systems listed in the summary table above were targets for toxicity in rats by 26 weeks.
B) Neurotoxicities (seen after 50-52 weeks of dosing) may be of concern for chronic dosing with FK506 in the clinic. Clinical monitoring should include periodic evaluations for all of the organs and systems listed. The kidney was a target for adverse effects of FK506 at the same dose and dosing periods as the immune system; therefore, renal toxicity is likely to be dose-limiting in the clinic.
C) Myocardial inflammation due to i.v. FK506 is not likely to present a clinical risk as this effect was seen in rats at 1X the recommended clinical dose; dosing with the i.v. form should not extend beyond 4 weeks unless more intensive i.v. animal testing is conducted.
D) While FK506 has been shown to be effective in preclinical models of graft rejection and autoimmune disease, no research has been conducted to evaluate circulating levels of FK506 or metabolites and correlate these levels with positive and negative therapeutic outcomes.
E) Very little nonclinical research has been conducted to evaluate the pharmacokinetics and in vivo metabolism of FK506: 1) following repeated dosing, or 2) in combination with other immunosuppressive agents and concomitant medications. Such studies are recommended to provide more accurate risk assessments; until this research is conducted, extrapolation of doses from nonclinical studies must utilize body surface area conversion.
F) At a dose in rats equivalent to 5X the recommended clinical dose, a marked shift in the distribution of FK506 was seen in blood compared with plasma; while this high a dose will not be administered to patients, higher than expected plasma levels might occur in patients with reduced FK506 clearance such as in cases of liver dysfunction.

Studies performed in baboons clarified the doses and target organs associated with adverse effects of FK506 given by oral gavage. In general, toxicity profiles of FK506 are very similar in baboons and rats; only in rats were decreased lymphocyte counts (which correlated with histopathologic indicators of immune suppression) seen. Results showed that baboons tolerated higher doses of FK506 (when doses were corrected for body surface area) than did rats; therefore, dose extrapolations based on doses in rodents will provide conservative assessments of clinical risk.

Reproductive toxicity: FK506 should be designated as "Pregnancy Category C."
FK506, at oral doses of 0.32 and 1.0 mg/kg during organogenesis in rabbits, was associated with maternal toxicity as well as an increase in incidence of abortions; these dose are equivalent to 1/3 and 1X (based on body surface area correction) the recommended clinical dose (0.3 mg/kg). At the higher dose only, an increased incidence of malformations and developmental variations was also seen. FK506, at oral doses of 3.2 mg/kg during organogenesis in rats, was associated with maternal toxicity and caused an increase in late resorptions, decreased numbers of live births, and decreased pup weight and viability. FK506, given orally at 1.0 and 3.2 mg/kg (equivalent to ½X and 1.5X the recommended clinical doses based on body surface area corrections) to pregnant rats after organogenesis and during lactation, was associated with reduced pup weights.

FK506, given orally at 1.0 mg/kg (½X the recommended clinical dose based on body surface area correction) to male and female rats prior to and during mating as well as to dams during gestation and lactation, was associated with adverse effects on female reproduction and embryolethality. Effects on female reproductive function (parturition) and embryolethal effects were indicated by a higher rate of pre-implantation loss and increased numbers of undelivered and nonviable pups. When given at 3.2 mg/kg (1.5X the recommended clinical dose based on body surface area

correction), FK506 was associated with maternal and paternal toxicity, as well as reproductive toxicity including marked, adverse effects on estrus cycles, parturition, pup viability, and pup malformations. Toxicities to parental rats were indicated by tremors and circling, as well as reduced weight gains and food consumption in males; and reduced food consumption during gestation and lactation in females. Adverse effects on reproductive parameters included 1) increased copulatory intervals, 2) increased pre- and post-implantation loss of fetuses (resulting in smaller litter sizes), and 3) decreased numbers of dams delivering. No reduction in male or female fertility was evident. Adverse effects seen in pups were markedly reduced viability and a slight increase in the incidence of malformation (3 pups from 3 dams).

Genotoxicity: No evidence of genotoxicity or clastogenicity was seen in bacterial (Salmonella and E.coli) or mammalian (chinese hamster lung-derived cells) in vitro assays of mutagenicity, the in vitro CHO/HGRPT assay of mutagenicity, or in vivo clastogenicity assays performed in mice; FK506 did not cause unscheduled DNA synthesis in rodent hepatocytes.

Human pharmacokinetics: Cytochrome p450 3A, as shown in in vitro assays using human liver microsomes, may be a crucial enzyme for metabolizing FK506 in humans. Erythromycin, fluconazole, and prednisolone may be important drugs for evaluating pharmacokinetics in combination treatment with FK506, as phase 4 commitments (see medical officer's review).

Carcinogenicity: No studies adequate to evaluate the carcinogenicity of FK506 have been submitted. Studies are currently underway in mice and rats.

Allergic potential: The potential for allergic reactions to HCO-60 should be mentioned in the package insert.

Lauren E. Black, Ph.D.
Reviewing Pharmacologist

Concurrences:
HFD-530/Dep/LRosenstein 12/16/93
HFD-530/SPharm/JFarrelly 12/14/93
Disk:
HFD-530/LRosenstein
cc:
HFD-530/NDAs 50-708 and 50-709
HFD-530/Division File, NDAs 50-708 and 50-709
HFD-340
HFD-530/LBlack
HFD-530/CBroadnax
HFD-530/MCavaille-Coll
HFD-530/MSeggal
HFD-345/GJames