

Pharm

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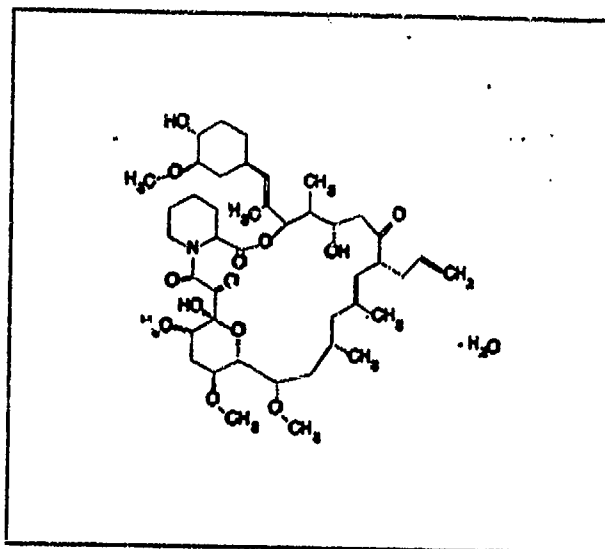
SPONSOR: Fujisawa
DRUG: FK506
ROUTE ADMINISTERED: oral tablet

RELATED INDs:

INDICATION: immune suppression of liver graft rejection

DEFINITIONS:

- FK506BP = FK506 binding protein
- FK506 = PROGRAF® = Tacrolimus
- 2-AAF = 2-acetylaminofluorene
- TC-5R = Hydroxypropylmethylcellulose 2910 (USP)
- Ac-Di-Sol = Croscarmellose sodium (carboxymethyl cellulose sodium, NF)
- CTL = cytotoxic T-lymphocyte
- PCA = passive cutaneous anaphylaxis
- ASA = active systemic anaphylaxis
- TNBS = trinitrobenzenesulfonate
- HCO-60 = polyoxyethylene hydrogenated castor oil
- WBC = white blood cell
- CyA = cyclosporin A
- LD = low dose
- MD = middle dose
- MST = mean survival time
- t_{max} = time until C_{max}
- V_d = volume of distribution
- F2 = second generation
- BUN = blood urea nitrogen
- TNF-α = tumor necrosis factor alpha
- MLR = mixed lymphocyte reaction
- IC₅₀ = concentration, 50% inhibition
- CII = bovine collagen
- HVG = host versus graft
- MCV = mean corpuscular volume
- GM-CSF = granulocyte-macrophage colony stimulating factor
- HPLC = high performance liquid chromatography
- Orthograft: substitute donor organ for recipient's
- Heterograft: add donor organ, without removing original organ.
- Allograft: donor and recipient are of the same species
- Xenograft: donor and recipient are of different species.
- LYM = lymphocytes
- ND = not determined
- HD = high dose
- IL = interleukin
- F1 = first generation
- t_{1/2} = half-life
- C_{max} = maximum concentration
- BWG = body weight gain
- LDH = lactate dehydrogenase
- CTL = cytotoxic T lymphocyte
- LD₅₀ = lethal dose, 50% of treated population
- PHA = phytohemagglutinin
- EAU = experimental autoimmune uveoretinitis
- GVHD = graft versus host disease
- MCHC = mean corpuscular hemoglobin concentration
- RBC = red blood cell
- IV = intravenous
- PO = perioral
- IM = intramuscular
- AUC = area under the curve



BACKGROUND

FK506, a macrolide similar in activity to Cyclosporin A, has been used in the clinic since early 1989 as an immunosuppressant therapy to prevent organ rejection in graft recipients. Organs which

have been successfully treated following transplantation have included kidney, liver, heart, lung, and pancreas. FK506 has been used in combination with other immunosuppressive agents. These include prednisone or other steroids, antilymphocyte immunoglobulin or OKT3, antiinflammatory agents such as prostaglandin E1, and anti-metabolites such as cyclophosphamide or azathioprine. These combinations rely on diverse mechanisms to suppress both cellular and humoral immune reactions to foreign tissue. FK506, like cyclosporin, has shown itself to be especially active in suppressing T cell responsiveness to novel antigens, and for this reason, FK506 is administered simultaneously with or prior to engraftment. Studies using cultured lymphocytes have documented that FK506 suppresses the mitogenicity of Concanavalin A, mixed lymphocyte responses, and the responses of cytotoxic T-lymphocyte precursors, as well as cellular production of IL-2, IL-2 receptor, IL-3, IL-4, γ -interferon, TNF- α , and GM-CSF. Because no direct effects of FK506 have been noted on intracellular signals evolved by T-cell receptor binding to antigen, interaction of FK506 at the level of gene regulation of IL-2 gene transcription have been implicated. At least 5 different proteins must bind to gene regulatory regions upstream from the IL-2 promoter region in order for the IL-2 gene to be transcribed. Interfering with the nuclear factors which bind to these regions is a likely explanation for the diminished production of IL-2 seen following FK506 treatment. A protein named "FK506-binding protein," (FKBP) an immunophilin which binds to FK506 with high affinity, is likely to be involved in mediating FK506's effects on gene transcription.

Clinical usage of FK506 is dictated by several factors including indications of drug toxicities at higher doses and the likelihood of rejection at lower doses. To complicate the picture, FK506 is rarely used clinically as monotherapy; FK506 is often combined with short-term steroids. FK506 treatment is started with i.v. infusions, with conversion to oral tablets, twice/day as soon as possible. In monitoring FK506 levels, whole blood rather than plasma should be used due to the sequestration of FK506, and possibly metabolites, in erythrocytes. The blood levels correlating with optimal clinical benefits, or the proposed efficacious range, is thought to be ng/ml . Doses are adjusted downwards with time following surgery to avoid toxicities (renal, neural, and carbohydrate metabolic), and to maintain sufficient immunosuppression to prevent graft rejection. For the 1st month, blood levels correlated with these doses are 10-25 ng/ml ; during months 2-3, levels are 5-20 ng/ml ; and during months 4-12, levels are 5-15 ng/ml . The oral doses needed to achieve these blood levels are not precisely identified in the submissions to date, but oral dosing is typically started at 0.3 mg/kg/day .

INTRODUCTION

The following comprises a review of the nonclinical toxicology studies performed with FK506, as well as summaries of additional pharmacology, nonclinical therapeutic, and pharmacokinetic reports submitted by the sponsor to complete the filing of the NDA. Previously reviewed material relevant to the NDA is contained in three reviews of the IND containing analysis of the rat, baboon, genotoxicity, and reproductive toxicity studies. The stamp dates of these reviews are 8/31/93, 9/20/93, and 9/22/93; one review is in final processing.

Comment: The review of the mouse dietary studies of FK506 (13-week dose ranging and 78-week carcinogenicity studies) is written, but is being considered separately from the NDA, as carcinogenicity studies were agreed by the agency and the sponsor not to be required for NDA approval. See the review labeled, "IND containing analysis of the rat, baboon, genotoxicity, and reproductive toxicity studies," for the discussion of these studies. The study performed in rats has not yet been submitted, and the mouse study is currently not considered adequate for the purposes of product labelling.

NONCLINICAL TOXICOLOGY STUDIES: OVERVIEW**Study Summary:****A. Acute**

- A1. Acute toxicity study of FK506 in rats following i.v. and oral dosing¹. (GLR880181; Fujisawa, non-GLP; 5/87-8/87; lot no. 016073L).
- A2. Acute toxicity of FK506 in young rats following oral dosing. (GLR910392; Fujisawa; Japan GLP; 5/90-9/90; lot no. 10306YL).
- A3. FK506 acute oral and i.v. toxicity to baboons by single administration.
Study no. 881636; lot # G02672S; GLP; 5/28/88)
- A4. Single dose toxicity study of FK506 and its deterioration products, related compounds, and tautomer in Jcl:ICR mice (i.v. dosing). (Fujisawa; Japan GLP; GLR 910601; 7/91; lot no. not provided)
- A5. Single dose toxicity study of related compound and a metabolite of FK506 in mice using i.v. dosing. (Fujisawa; Japan-GLP; lot no. not provided ; GLR920309; 1/92)

B. Subchronic

- B1. Preliminary 2-wk oral toxicity study of FK506 in rats. (GLR 910477; Fujisawa; non-GLP; 2/86-4/86; lot no. 011050L).
- B2. FK506 toxicity to rats by repeated oral administration for 13 weeks. (GLR 880273; GLP; 10/26/87-1/26/88; lot no. 10306YL)
- B3. FK506 toxicity to rats by repeated oral administration for 52 weeks. (GLR 910589; GLP; 2/12/90-2/11/91; lot no. s 034103L, 8/27/90-2/8/91; and 018270L, 2/6-8/24/90)
- B4. Four-week oral toxicity study of FK506 in young rats. (GLR 910393; Fujisawa; Japan GLP; 7/90-3/91; lot no. 018178L)
- B5. FK506 toxicity to rats by repeated i.v. administration for 4 weeks. (GLR 900160; GLP; 12/8/88-1/5/89; lot no. 018178L).
- B6. FK506 preliminary toxicity study in baboons by repeated oral administration for 28 days.
Study no. 880271; 6/24/87; GLP; lot no. G00371S)
- B7. FK506 toxicity to baboons by repeated oral administration for 13 weeks.
; Study no. 880272; GLP; lot no. G02672S; 11/4/87)
- B8. FK506 toxicity to baboons by repeated oral administration for 13 weeks II.
Study no. 890443; GLP; lot no. G02672S; 7/15/88)
- B9. FK506 toxicity to baboons by repeated administration for 52 weeks.
Study no. 910520; GLP; lot no. 239684K; 3/13/91)
- B10. FK506 toxicity to baboons by repeated i.v. administration for 4 weeks.
Study no. 890444; GLP; lot no. 111385K, 111285K, and 111185K; 8/11/88)
- B11. Four week i.v. toxicity of FK506 in rabbits with 4-wk recovery. (GLR 930031; non-GLP; lot no. 718619K; 4/2/92)
- B12. Dose-range finding study in rats by dietary administration for 13 weeks.
Lot no. 203001K; GLR 910196; GLP; 3/23/90)

¹Unless otherwise stated, FK506 was administered by oral gavage to animals used in all toxicity studies in a dispersed suspension, stirred continuously during dosing. The formulation for the vehicle was TC-5R, lactose, and Ac-Di-Sol at proportions of 1:2:1, stirred with distilled water. The formulation used for i.v. dosing in nonclinical toxicity studies was HCO-60, polyoxyethylene hydrogenated castor oil.

B13. Preliminary 2-week oral toxicity study of FK506 in dogs. (Fujisawa; GLR 910395; Lot no. FR011050L; 4/86; non-GLP)

C. Genotoxicity

C1. Evaluation of FK506 in a chromosomal aberration test with chinese hamster lung cell line V79. (Fujisawa; GLR 930182; Japan GLP; lot no. 0541YL; 4/93)

C2. Evaluation of the potential of FK506 to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F-344 rat. (GLR 910559; GLP; 5/20/91-8/8/91; lot no. 038008L).

C3. FK506 effects on in vitro non-mammalian cell systems: I. (Reversion or Ames tests). (GLR 880249; Fujisawa; 9/87; lot no. 011050L; Japan GLP)

C4. FK506 effects on in vitro non-mammalian cell systems: II. (Reversion or Ames tests). (GLR 910396; Fujisawa; 10/90; lot no. 036005L; Japan GLP)

C5. Mutagenicity study of FK506- chromosomal aberration test with chinese hamster lung cells in culture. (GLR 880250; Fujisawa; 9/87; lot no. 011050L; Japan GLP)

C6. Evaluation of FK506 in the chinese hamster ovary cell/HGPRT gene mutation assay. (GLR 910560; 4/91; lot no. 038008L; GLP)

C7. Mutagenicity study of FK506- micronucleus test in mice following single oral dosing. (GLR 910320; Fujisawa; 8/90; lot no. 018178L; Japan GLP)

C8. Mutagenicity study of FK506- micronucleus test in male and female mice. (GLR 930076; Fujisawa; 11/92; lot no. 207781K; Japan GLP and OECD)

D. Reproductive toxicity

D1. Study of FK506 on fertility and general reproductive performance in rats (Segment I) (GLR890455; GLP; lot no. 018178L and 014071L, 5/11/88-9/7/88)

D2. Developmental toxicity study in rats of p.o. FK506 (Segment II) (GLR910516; GLP; lot no. 018270L; 4/17/90-9/29/90)

D3. Perinatal and lactation study of FK506 in rats (Segment III) (GLR91059; GLP; lot 034103L; 11/2/90; I)

D4. Segment II reproductive toxicity study in New Zealand white rabbits. (GLR 890389; GLP; 8/1/88; lot no. 018178L)

E. Vehicle toxicity

E1. Reactions to HCO-60 (GLR920256; non-GLP;

E2. Single dose toxicity study of HCO-60 in rats by i.v. dosing. (Fujisawa; GLR930079; non-GLP; 11/92; lot no. FF1853)

E3. HCO-60 toxicity to rats by repeated administration for 4 weeks. (GLR910360; non-GLP;

E4. (34,654/151) Four week i.v. toxicity study of HCO-60 in rats. (Fujisawa; GLR 930211; Japan GLP; lot no. FF-2198; 1/93)

E5. (34,654/151) Mutagenicity of HCO-60 (micronucleus test) in mice. (Fujisawa; GLR 930173; Japan GLP; lot no. FF-2918; 2/93)

E6. (34,654/151) Evaluation of HCO-60 in chromosomal aberration test with chinese hamster lung cells line V79. (Fujisawa; GLR 930167; Japan GLP; lot no. FF-2918; 2/93)

E7. Antigenicity study of HCO-60 and cremophor in guinea pigs. (Fujisawa; GLR920248; Japan-GLP; lots FF0733 and MOE1381; 8/3/90)

E8. Correlation between plasma histamine levels and infusion time of HCO-60 in dogs. (Fujisawa; non-GLP; lot no. FF0733; 7/92; GLR 920324)

E9. HCO-60 toxicity to baboons by repeated i.v. administration for 4 weeks.
Study no. 910404; non-GLP; lot no. 111585K; 6/28/88)

E. Special toxicity

F1. Morphological and functional changes in islet of Langerhans in FK506 treated rats.
(GLR910177; non-GLP; Fujisawa)

F2. Effects of FK506 and cyclosporin A on exocrine function of the rat pancreas. (GLR910616;
non-GLP; Fujisawa)

F3. Antigenicity study of FK506 in mice. (Fujisawa; Japan-GLP; GLR910600; lot no. 109003K;
8/91)

F4. Antigenicity study of FK506 in guinea pigs. (Fujisawa; Japan-GLP; GLR910318; 10/88; lot no.
019186L)

F5. Local irritation test of FK506 injectable formulation in rabbits by i.m. injection. (Fujisawa; GLR
890179; Japan-GLP; lot no. 111685K; 11/88)

TOXICITY STUDY REVIEWS

ACUTE TOXICITY:

A1. Acute toxicity study of FK506 in rats following i.v. and oral dosing. (GLR880181) Sprague-Dawley rats (5/sex/group) were given i.v. doses of vehicle (PEG-400), or FK506 at doses of 10, 32, or 100 mg/kg; or oral doses of oral vehicle, or FK506 doses of 32, 100, or 180 mg/kg. Animals were observed twice per day and weighed 5 times following the dosing day until day 14. The LD₅₀ was calculated by probit method for i.v. dosing to be 57 mg/kg for males and 24 mg/kg for females; similarly for oral dosing, 134 mg/kg for males and 194 mg/kg for females. Survivors (of doses which were lethal to some animals) showed transient reductions in weight gain which had normalized by the end of day 7. Rats died in 5 min following i.v. doses of 100 mg/kg; deaths following 32 mg/kg tended to fall within the next 5 days. Autopsies of the dead animals revealed no definite cause of death. Clinical signs were evident following all i.v. doses in males- bloody urine at 10 mg/kg, prostration up to 30 min post-dose, and reduced activity up to 6 h post-dose at 32 mg/kg. In females, the same signs were noted, but additionally, ptosis and hypersensitivity to touch were noted shortly prior to deaths. Bloody urine was not noted following oral doses of FK506; no signs were noted on day 1 following oral doses, and signs (same as those above) noted on days 2 and later were shortly prior to death. Necropsy findings of animals dead on study following i.v. or oral doses revealed red or black foci in the glandular stomach (correlating with submucosal hemorrhage), atrophied spleen and thymus, and intussusception of the jejunum and ileum.

A2. Acute toxicity of FK506 in young rats following oral dosing. (GLR910392) Sprague-Dawley rats (age 21 days, weight approximately 50 g, 5/sex/group) were given oral vehicle or FK506 at a dose of 0, 10, 32, 100, or 320 mg/kg. Mortalities were seen at doses at and above 32 mg/kg on days 1-6. The LD₅₀ calculated from probit analysis was approximately 70 mg/kg. Abnormal clinical signs were seen later than 6 h post-dosing on day 1 and days 2-10 in surviving animals; these included salivation, increased irritability, and decreased activity. Suppressed body weight was noted on day 2 at 32 mg/kg in females and at 10 mg/kg (and above) in males. The rats found dead generally had atrophied lymphoid follicles in the spleen and thymus, red foci (erosion) in the glandular stomach, ulcer in the forestomach, and thick limiting ridge. The exact cause of death was not determined.

A3. FK506 acute oral and i.v. toxicity to baboons by single administration. FK506 was administered i.v. to 2 baboons (1 male, 1 female) and orally to 2 baboons. Single, ascending doses

were given with 2-week washout periods. Baboons were given 50, 100, or 250 mg/kg of oral FK506, or vehicle, 2, 10, or 50 mg/kg of i.v. FK506. Clinical signs seen following administration of drug were quietness, huddled posture, and piloerection. Following 50 mg/kg i.v., one female was sacrificed for humane reasons; an acute shock reaction (flushing, prostration, hypotension) followed the injection; the animal showed unsteadiness and dyspnoea; 5 h later, no improvement was seen and sacrifice was conducted. The male receiving the same dose showed similar signs, but recovered fully after 4 days. Administration was associated with reduced RBC, PCV, and hemoglobin only at 250 mg/kg orally and 2 mg/kg i.v. (and higher; the response did not change with dose). Anemic changes were evident 7-10 day following the dose. No control animals were allotted to this study; therefore, comparison of organ weight changes were not conducted. No histopathological analysis was conducted.

A4. Single dose toxicity study of FK506 and its deterioration products, related compounds, and tautomer in Jcl:ICR mice (i.v. dosing). Single i.v. doses of products related to FK506, as well as light (exposed to 30,000 lux for 14 days) and heat damaged vehicle and FK506 (exposed to 90 degrees C for 10 days) were administered at 56 and 100 mg/kg to mice (5/sex/group). FK506 was administered at doses of 18, 32, 56, and 100 mg/kg. The LD₁₀₀ for all the test substances was 100 mg/kg; animals died within 5 minutes. One death (out of 10 mice) was observed for FK506 (undamaged) at 56 mg/kg, but 7/10 deaths were observed with light-damaged FK506 at this dose. Adverse clinical signs were seen at 32 mg/kg of FK506, including decreased spontaneous motility and prone position. Necrosis and loss of part of the tail were seen at doses of 5.6 mg/kg and above following injection in the tail.

A5. Single dose toxicity study of compounds related to and a metabolite of FK506 in mice using i.v. dosing. Single i.v. injections of 56 or 100 mg/kg were injected into Jcl:ICR mice (10/sex/group) to evaluate doses associated with lethality. No significant differences in mortality due to injection of these compounds were noted.

SUBCHRONIC TOXICITY:

B1. Preliminary 2-wk oral toxicity study of FK506 in rats. (GLR 910477) Sprague-Dawley rats (6-week-old; 5 males/group) were used to in a dose-ranging study to identify the doses for a 13-week study; histopathological analysis was limited to liver, kidney, spleen, and thymus. Rats were given oral vehicle or FK506 at doses of 1.0, 3.2, 10, or 32 mg/kg of FK506 daily for two weeks. Effects were compared with a positive control group, cyclosporin (in olive oil) at doses of 32 or 100 mg/kg. While no mortalities were seen on study, weight loss was seen in the 32 mg/kg group, and decreased body weight gain (23%) was seen at 3.2 mg/kg; food consumption was not affected by FK506 (but was reduced by 100 mg/kg cyA). At 3.2 mg/kg, slight increases in creatinine and potassium, and decreases in sodium and calcium were seen (or at 32 mg/kg of CyA) as well as decreased spleen and liver weights. At 10 mg/kg, urine output (measured post dosing) decreased from 3.0 to 1.8 ml; glucose was also noted in the urine (as well as at 32 mg/kg of cyA), increased cholesterol and triglycerides were seen (as well as at 32 mg/kg of cyA), and decreases were noted in organ/body weight ratios of the liver, spleen and thymus (atrophy). At 32 mg/kg, increased reticulocytes, neutrophil, and eosinophil counts, decreased RBC and hemoglobin, total protein and albumin, and increased BUN and kidney weight/body weight ratios were seen with tubular atrophy and calcification.

B2. FK506 toxicity to rats by repeated oral administration for 13 weeks. (GLR 88-273) Sprague-Dawley rats (5 weeks old; 12/sex/group) were given oral vehicle or FK506 at doses of 0.32, 1.0, or 3.2 mg/kg (groups 1-4) daily for 13 weeks. An additional group was given vehicle (olive oil) or CyA at 10 mg/kg. One mortality was seen on study due to anesthetic overdose following

hematologic sampling during week 13; no mortalities occurred which were attributable to drug exposure. The only clinical sign seen on study was occasional post-dosing salivation, evident at a low incidence in all dosed groups. Effects in group 4 included decreased body weight gain (males; slight effect in females), slight reductions in food consumption in week 1, reduced efficiency of food utilization (males) towards the end of dosing, increased water consumption (males), and lens opacity (or lenticular degeneration; 3 /12). Serum chemical and hematological changes seen in group 4 included decreased packed cell volume and MCV as well as increased MCHC; decreased lymphocytes and WBC (males); increased BUN, cholesterol and phospholipids; increased urea nitrogen; decreased total proteins and globulin, decreased glucose (males), glucose in urine (males), and slightly decreased potassium (males), slightly increased LDH (females); and acidified urine. Effects on organ weights and histopathology included decreased weights of male reproductive organs, spleen, submaxillary glands, and liver; increased ovary weights, increased cervical lymph node size, decreased thymus cellularity with cortical lymphocytolysis, pancreatic vacuolation (males), basophilic cortical tubules (group 3 as well), mineralization of the corticomedullary junction of the kidney; focal encephalomyelitis of the brain and spinal cord, and interstitial inflammation of the Harderian gland (males). (Effects seen with CyA were decreased thymus cellularity, hyperplasia of the cervical lymph nodes, decreased prostate weights, increased ovary weights, focal encephalomyelitis, slight decreases in lymphocyte count, increased glucose excretion, and decreased body weight gain in males.)

B3. FK506 toxicity to rats by repeated oral administration for 52 weeks. (GLR 910589) Sprague-Dawley rats (20/sex/group) were given daily oral vehicle or FK506 at doses of 0.15, 0.5 or 1.5 mg/kg groups 1-4) of FK506. Mortalities occurred among 1 male in group 2 (unknown cause), 1 male in group 3 (brain tumor - astrocytoma), 6 males in group 4 (2 with nerve lesions, 1 in poor clinical condition, 3 unknown causes), 1 female in group 1 (pituitary tumor), and 1 female in group 3 (lymphoma - see comment 1 below). Many group 4 males exhibited reduced body weight, tremors, unsteadiness, and poor condition of coat. Other animals exhibited hunched posture as well as these clinical signs. Observations included reduced body weight gains (groups 3 and 4 males, week 0-1; group 4 males, weeks 1-26), weight loss (group 4 males, weeks 26-52), reduced food consumption (groups 3 and 4 males, week 0-1; group 4 males, weeks 26-52); slightly reduced food utilization efficiency (group 4 males and females, weeks 2-26), greatly increased water consumption (group 4 males, week 52) and cataract (1 male, week 52).

Hematological findings were reduced WBC and lymphocytes (groups 3 and 4 males, week 13 and females, week 26), increased hemoglobin, MCHC, and MCH (week 26, group 4 males), and increased neutrophils and eosinophils (group 4 males, week 52). Serum chemical changes noted were decreased albumin (group 4 males, week 13), increased plasma urea nitrogen (groups 3 and 4 females, week 13; groups 4 males, week 26), increased phospholipid and triglycerides (group 4 females, week 13; group 3 females, week 26; groups 3 and 4 males, week 26), decreased potassium and increased sodium (group 4 males, week 52) as well as increased alkaline phosphatase (group 4 females, week 26; groups 3 and 4 males, week 52), GOT, and GGT (group 4 males, week 52). Urinalysis revealed reduced urinary protein (group 4 males, week 13), decreased pH in males (group 4, week 13), increased specific gravity (group 4 males, week 26), increased total reducing substances (sugars) were evident (group 4 males, high incidence, and females, low incidence, week 26).

Pathology results in group 4 indicated increased kidney/body weight ratios and decreased prostate ratios (males), increased thymus, uterine, ovarian, and kidney weight ratios (females), reduced thymus size (males), cortical/subcapsular lens opacity (13/14 group 4 males; 1 group 3 and 2 group 4 females; week 52 only), and reduced adipose tissue. Microscopic analysis confirmed

reduction of thymic medulla, vacuolation and hypertrophy of pancreatic islet cells, clear cells in kidney collecting tubules, reduced colloid in prostate and seminal vesicles, luminal dilatation in uterus, decreased incidence of corpora lutea in ovaries, lenticular degradation and degenerative fibres (with prominent gitter cells) in eyes, degenerative fibers in sciatic nerve (2 group 4 males, which exhibited loss of hindlimb use and subsequently were sacrificed in moribund condition; 5 males and 1 female in group 4 which survived to study termination), and inflammation in spinal nerve root (1 female, group 3). Distension (nongaseous) of the stomach, small intestine, and cecum was evident in group 4 males and females; corpus mucosae were congested in some males of group 4.

Comment 1: Female no. 122 died on week 38, showing the following clinical signs: loss of hindlimb use, red staining of muzzle, pallor of extremities, red periorbital staining. On microscopic evaluation, lymphoblastic lymphoma was noted with lymphoid multicentric tumors. Lymphocytolysis was noted in the thymus. Transformed lymphocytes were evident in lymph nodes, liver, spleen, ovaries, skeletal muscle, bone marrow, femur joint. Also evident was degenerative nerve damage in spinal cord and spinal nerve roots of the lumbar region.

Comment 2: Following evaluation of individual animal data, no direct (1:1) correlation could be drawn between degeneration of sciatic nerves and unsteady gait, as some animals which evidenced altered gait had no evident nerve degeneration. Similarly, no 1:1 correlation could be drawn between fasted blood glucose levels and any of the following: poor clinical condition, lenticular degeneration or vacuolated pancreatic islet cells. In both cases, the incidence of these findings increased with dose. Interestingly, there was limited histopathologic evidence of immune suppression - decreased WBC and reduction in thymic medulla; there were no decreases in size or number of germinal centers in the white pulp of the spleen or lymph nodes.

B4. Four-week oral toxicity study of FK506 in young rats. (GLR 910393) 21-day old rats were given oral vehicle or FK506 at doses of 0.32, 1.0, and 3.2 mg/kg. No changes were evident in survival, clinical signs, ophthalmic parameters, body weights, or food consumption. Hematological changes evident at 3.2 mg/kg were slightly lower WBC and lymphocyte counts, and slight drops in hemoglobin counts (males). Dose-related serum chemical changes evident at 3.2 mg/kg were increased serum cholesterol (males), slightly increased glucose, creatinine, and BUN; as well as increased inorganic phosphate, sodium, calcium, and decreased chloride (males). Decreased pituitary, salivary gland, spleen (slightly), and increased ovary weights were seen. Histopathological changes noted were slight hypoplasia in lymphoid follicles of the spleen, cortical thickening in the thymus, moderate degree of calcification in the outer medulla of the kidney, and basophilic tubules in the kidney.

SUMMARY OF TOXICITY STUDIES: ORAL DOSING WITH FK506 IN SPRAGUE-DAWLEY RATS

Dose, mg/kg	Acute		Preliminary - 2 week		Subchronic - 3 month		Chronic - 1 year	
	Male	Female	Male	Female	Male	Female	Male	Female
0.15	ND		ND		ND		NOEL	
0.32	ND		ND		NOEL		ND	
0.50	ND		ND		ND		weeks - 13: †WBC; 52: †alkaline phosphatase; †BWG and food consumption week 26: †phospholipids, triglycerides	weeks - 13: †BUN; 26: †WBC 52: lymphoma (1)
1.0	ND		NOEL		lenticular degeneration basophilic cortical tubules		ND	
1.5	ND		ND		ND		weeks - 13: †urinary protein & pH, †albumin; 26: †urine specific gravity, urine glucose, BUN; †hemoglobin, MCHC, & MCH; 52: †thymus weight; †Na, †K; †neutro. & eosinophils †GOT, GGT; †BW, †water intake, lens opacity (all), cataract (1); tremors, unsteady gait, hunched posture; † prostate/BW, colloid in prostate and sem. vesicles †prominence of thymic medulla; †kidney weight, clear cells in kidney tubules; †total proteins and albumin; vacuolated pancreatic islets, lenticular degeneration; degeneration of sciatic nerve; †food utilization, nongaseous distended bowels, congested corneal mucosa	weeks - 13: †phospholipids, triglycerides; 26: †alkaline phosphatase. 52: † thymus weight; † ovary & uterine weights; † absence of corpora lutea; luminal dilation in uterus

SUMMARY OF TOXICITY STUDIES: ORAL DOSING WITH FK306 IN SPRAGUE-DAWLEY RATS - CONTINUED

Dose, mg/kg	Acute		Preliminary - 2 week	Subchronic - 3 month		Chronic - 1 year	
	Male	Female		Male	Female	Male	Female
3.2	ND		↓BWG; ↑creatinine; ↑Na & Ca; ↑K; ↑spleen and liver weights	↓BWG, food utilization efficiency; ↓LYM, WBC; ↑water intake; lens opacity pancreatic vacuolation, ↓glucose, glucose in urine; ↓weight of reproductive organs ↑cervical lymph node wt; ↓thymic cellularity; ↓ spleen weight; ↑BUN, ↓urine pH, mineralization of corticomedullary junction in kidney; ↑cholesterol, phospholipids; ↓TP, globulin, & liver weight; ↑MCV & MCHC; ↓submaxillary gland weight; inflammation of Harderian gland; focal encephalomyelitis in brain	↑LDH; ↑ovary weight	ND	
10.0	NOEL		↑urine output, ↑glucose in urine; ↑organ/BW of liver, spleen, and thymus	ND		ND	
32.0	NOEL	NOEL	weight loss; ↑BUN and kidney/BW; ↑total proteins and albumin; ↑hemoglobin and RBC; ↑reticulocytes, neutrophils & eosinophils	ND		ND	
100	LD ₅₀ = 134	LD ₅₀ = 190	ND	ND		ND	
320			ND	ND		ND	

EFFECTS OF ORAL FK506 IN 21-DAY-OLD SPRAGUE-DAWLEY RATS				
Dose (mg/kg)	Single dose study		Repeated dose study - 28 days	
	Male	Female	Male	Female
0.32	ND		No effects	
1.0	ND		NOEL	
3.2	ND		↑ phosph., ↑ Na & Ca, ↓ Cl; ↑ cholesterol; ↓ hemoglobin	↑ ovary weight
			↓ WBC, LYM, & spleen weight; hypoplastic lymphoid follicles, cortical thickening in thymus; ↑ creatinine & BUN; calcified outer kidney medulla, basophilic tubules; ↑ glucose, ↓ pituitary & salivary gland wts.	
10.0	↓ BW, day 2	NOEL	ND	
32.0	Minimum lethal dose	Minimum lethal dose; ↓ BW, day 2	ND	
100	LD ₅₀ = 70 mg/kg		ND	
320	lethal		ND	

B5. FK506 toxicity to rats by repeated i.v. administration for 4 weeks. (GLR 900160) FK506 was administered by daily i.v. injections of vehicle (400 mg of HCO-60 in 1 ml of ethanol, diluted 1:50 with saline solution) or FK506 at doses of 0.032, 0.1, 0.32, or 1.0 mg/kg to Sprague-Dawley rats (12/sex/dose). Mortalities occurring on study (weeks 1, 3 and 4) were 1 female in the 0.1 mg/kg group (#92; death likely anesthetic overdose) and 2 females in the 0.32 mg/kg group (#s 102 & 108). Histopathologic analysis of these 3 animals revealed no more severe lesions attributable to drug than others in their dosing groups, and therefore, the relationship of these mortalities to drug treatment is uncertain. Effects on weight gain and food conversion were evident in males only, including slight decreases in body weight gain (BWG; 0.1 mg/kg), marked decreases in BWG (0.32 and 1 mg/kg), and decreased food conversion efficiency (weeks 1 and 2, 0.32 and 1.0 mg/kg; weeks 3 and 4, 0.1 mg/kg). Hematological and serum chemical findings were decreased WBC, lymphocytes (0.1 mg/kg in males, 0.32 in females); decreased neutrophils and eosinophils (↑ mg/kg); decreased total protein and albumin (0.32 mg/kg), increased BUN and potassium (1 mg/kg); increased triglycerides, cholesterol, and phospholipids (0.1 mg/kg, males), and increased cholesterol and phospholipids (.32 mg/kg, females). Urinalysis results showed decreased pH in urine (males, all doses), decreased urine protein concentration (0.1 mg/kg, males; or 1.0 mg/kg, females), and increased urine volume (1 mg/kg). The following changes in absolute organ weights were seen in males: decreased pituitary, thymus, spleen, prostate, testes, epididymides, and salivary gland weights, and increased lung, kidney, and adrenal weights (0.32 mg/kg). In females, decreased pituitary and spleen weights (0.32 mg/kg), and decreased uterine, ovarian, and kidney weights (1 mg/kg) were seen; dose-dependent decreases in salivary gland weight were evident at all doses (8-27%).

EFFECTS OF FK506 IN SPRAGUE-DAWLEY RATS FOLLOWING I.V. ADMINISTRATION				
Dose mg/kg	Single Dose		Repeated Dose - 4 Week.	
	Males	Females	Males	Females
0.032	ND		↑urine pH	↓WBC & LYM; mineralization of kidney corticomedullary junction; ↓salivary gland wt.
0.1	ND		↓BWG, food utilization efficiency; ↓urine protein; mineralization of kidney corticomedullary junction; ↓WBC & LYM; ↑triglycerides, phospholipids, & cholesterol	
			↓size and number of germinal centers, spleen and lymph nodes; ↓prominence of thymic medulla; vacuolated pancreatic islets	
0.32	ND		↓thymic, salivary gland weights; ↓male reproductive organ weights	↑cholesterol and phospholipids; narrow uterine walls
			↓pituitary and spleen weights; ↑lung and adrenal weights; ↓total protein and albumin	
1.0	ND			↓uterine, ovary and kidney wts.; ↓urine protein
			↑BUN, K, and urine volume; ↑neutrophils & eosinophils; perivascular inflam. at injection site; myocardial inflammation	
10.0	bloody urine		ND	
32.0	prostration, 6 h; LD ₅₀ = 57 mg/kg.	LD ₅₀ = 24 mg/kg; ptosis, hypersensitivity to touch	ND	
100	lethal, 5 min		ND	

Microscopic histopathological analysis revealed myocardial inflammation (minimal to moderate; 2/12 male and 1/12 female rats, 1.0 mg/kg), increased cortical lymphocytolysis and reduced prominence of medulla in the thymus (0.1 mg/kg), inconspicuous germinal centers in the lymph nodes and spleen (0.1 mg/kg), vacuolation of islet cells in pancreas (12/12 males and 1/12 females, 0.1 mg/kg), focal mineralisation in the corticomedullary junction of the kidney (0.1 mg/kg, males; 0.032 mg/kg, females), narrowed uterine walls (0.32 and 1.0 mg/kg), contraction of prostate and seminal vesicles (0.32 mg/kg), and inflammation and hemorrhage at injection sites (minimal degree, 1.0 mg/kg).

B6. FK506 preliminary toxicity study in baboons by repeated oral administration for 28 days. Groups of 2 baboons (1 male, 1 female) were given doses of vehicle, 3, 6, and 10 mg/kg/day of FK506 orally for 28 days. Slight losses of body weight and clinical signs (quietness, huddled posture, and piloerection) were seen at 10 mg/kg. No other effects were seen in hematological, serum chemical, or histopathological analysis.

B7. FK506 toxicity to baboons by repeated oral administration for 13 weeks. Groups of 6 animals (3 males, 3 females) were given doses of vehicle or 1, 3, 6, or 9 mg/kg/day of FK506 for 13 weeks. Quietness and huddled posture were seen at all doses. Body weight loss was seen in the

6 and 9 mg/kg groups in the first 2 weeks, followed by decreased body weight gain. Food consumption drops were seen in the first 3 weeks (females only), increased eosinophils, and decreased RBC (3 mg/kg) were seen. Decreased MCH, hemoglobin, serum calcium, total protein, and albumin and increased serum urea and glucose in urine were seen (6 mg/kg). Increased spleen weights, indistinct medulla of the thymus and inconspicuous germinal centers of the spleen were noted (6 mg/kg). Increased LDH and MCHC, as well as decreased PCV, MCV, total WBC and neutrophils were seen (9 mg/kg). No effects were seen on ophthalmoscopic or electrocardiographic parameters.

B8. FK506 toxicity to baboons by repeated oral administration for 13 weeks (II). A second 13-week study was performed with higher doses; groups (3 males, 3 females each) were given 0, 18, or 36 mg/kg/day. Clinical signs were lethargy, weakness, tremors, gingivitis, intermittent emesis or inappetance, and weight loss with subsequent suppression of body weight gain was seen in both treated groups. While increased APTT, decreased neutrophils, lymphocytes, platelets and erythrocytic parameters (decreased PCV, hemoglobin, RBC, MCHC, MCV, and MCH) were seen only at 36 mg/kg, the following effects were seen at both 18 and 36 mg/kg: increased serum urea, glucose, triglycerides, and urinary glucose and pH; and decreased cholesterol, albumin, phosphorus, and albumin/globulin ratio. Changes evident in necropsy results at 18 mg/kg were decreased thymic and pancreas weights; changes evident at 18 mg/kg in histopathological reports were decreased numbers of medullary lymphocytes of the thymus, and proliferation of large lymphocytes in the paracortical region of the lymph nodes, reduced germinal centers, thymic atrophy, reduced cellularity of lymphoid follicles in the intestinal lymphatic tissue, degranulation of exocrine cells of the pancreas, increased eosinophilic granularity in islets, lymphocytic infiltration of the intestines (36 mg/kg only), and interstitial inflammatory infiltrate with basophilic and dilated tubules (1 male, 36 mg/kg). No effects were seen on ophthalmoscopic or electrocardiographic examinations.

B9. FK506 toxicity to baboons by repeated administration for 52 weeks. Baboons (4/sex/group) were given oral vehicle or doses of FK506 at 1, 3.2, or 10 mg/kg/day. Effects at 10 mg/kg for 52 weeks were qualitatively and quantitatively the same as those seen at 9 mg/kg for 13 weeks with the following additional effects: at 10 mg/kg; increased spleen weight, increased myeloid cells in the bone marrow (1 male), and degranulation of exocrine cells of pancreas (seen at 18 mg/kg for 13 weeks) were seen. In all dosed groups, some animals showed discolored and congested gastrointestinal mucosae, one female at 1 mg/kg and 1 male at 10 mg/kg showed red discoloration of the rectum and passed occasional red stained faeces. The only hematological changes were slightly increased eosinophils at 10 mg/kg, 52 weeks; only 1 male at 10 mg/kg showed glucose in the urine.

B10. FK506 toxicity to baboons by repeated i.v. administration for 4 weeks. Baboons (3 males and 3 females/group) were administered i.v. vehicle or FK506 at doses of 0.5, 1, or 2 mg/kg/day (actually FK506 was given at 0.55, 1.1, and 2.2 mg/kg/day due to error in dilutions) for 28 days. One baboon (male, group 4) was seen to be ill, and was sacrificed due to humane reasons - on necropsy, in addition to the effects seen in other baboons, this male showed lesions and hemorrhage in the gastrointestinal tract caused by a fungal infection (perhaps secondary to immune suppression). Clinical signs were drowsiness, lethargy, pilo-erection, and hunched posture; all were seen in all treated groups. Slight losses of body weight (<10%) and decreased food consumption were seen in all groups. Glucose was elevated in the urine of all groups, while serum urea and potassium were elevated at 1 mg/kg and above. Increased BUN was seen at 1 mg/kg and above; a slight increase in urine pH was evident at 2 mg/kg. Hematological changes evident were slight decreases in WBC and neutrophils (1 mg/kg) and increased eosinophils (all treated groups). Platelet counts were down 27% in the 2 mg/kg group. Histopathological changes evident in all treated

groups (incidence increased between 0.5 and 1 mg/kg; no further increase was seen between 1 and 2 mg/kg) were adverse effects in organs of immunity (decreased germinal centers in spleen and lymph node, decreased numbers of non-germinal lymphocytes in spleen, increased apoptosis and indistinct medulla of thymus) as well as angiectasis in some islets of Langerhans of the pancreas. Increased prostate weight was seen at 2 mg/kg, and increased heart and lung weights were seen in all treated groups.

Comment: Immune suppression was evident at all doses, and was likely to have been the primary cause of the mortality seen on study (sacrifice for humane reasons, due to fungal infection). Pancreatic toxicity was seen at all doses as well, and evidenced as glucose in the urine (and possibly as angiectasis in pancreatic islets). Slightly decreased WBCs were seen at doses of 1 mg/kg and above. Kidney toxicity was evidenced as elevated BUN (1 mg/kg) and increased urine pH (2 mg/kg). No histopathological changes were noted in the kidney. No NOEL was determined.

B11. Four week i.v. toxicity of FK506 in male rabbits with 4-wk recovery. Rabbits (10/group; with 2 recovery groups, vehicle and high dose, 10 rabbits each) were given saline, vehicle, or daily i.v. doses of FK506 at 0.05, 0.1, or 0.2 mg/kg for 4 weeks. Recovery groups were necropsied after a further, 4 week, drug-free period. Four mortalities occurred on study, all in the 0.2 mg/kg group on days 18, 20, 21, and 28; 3/4 animals showed retention of fluid in the thoracic or abdominal cavity. Six of the remaining 8 animals in this high dose groups showed retention of fluid in the pericardiac cavity. At this high dose, reversible decreases were noted in HB, HC, and MCV; also decreases in sodium and chloride; increases in reticulocyte percent and potassium. The changes in electrolytes at this dose were probably indicative of adverse effects on kidney tubule function. At 0.1 and above, body weight loss was evident, as was an increased incidence of perivascular interstitial edema and fibrosis, thickening of arterial walls, and myofiber disarray. These effects were not evident in recovered rabbits, with the exception of slight increases in heart weight. While 2 rabbits in the 0.1 mg/kg groups showed some renal tubule degeneration, this effect was not seen in the high dose group. At 0.05 mg/kg, the following effects were seen: decreased body weight gain and food consumption; and increased serum glucose and heart/body weights.

Comment: 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 above, and decreased body weight gain and increased blood glucose at 0.05 mg/kg and above. 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.

B12. Dose-range finding study in rats by dietary administration for 13 weeks. Sprague-Dawley rats (10/sex/group) were given FK506 in the diet at doses of 0 (vehicle), 0.5, 2.5, or 5 mg/kg for 13 weeks. An additional set of animals (9/sex/group) were given the FK506 doses for the sole purpose of obtaining plasma samples at 9 a.m. on day 2 and weeks 6 and 12. No adverse clinical signs or mortalities occurred, nor were there any differences among the groups on study for food consumption or body weight gain measures. Levels at week 6 in males/females, from low to high doses, respectively, were 0/0.02, 0.03/0, and 0.08/0 ng/ml. Standard deviations equalled or exceeded these values, showing the plasma levels to be variable, and too low for useful comparison of dose proportionality. Hematologic measure showed only a slightly lower eosinophil count in all females, which was within expected normal values. A slight reduction in WBC was seen in high dose females, only. Slight reductions in mean calcium and phosphorus values in all treated males and (phosphorus only) in high dose females. While individual values were generally within normal ranges, 5 mg/kg is probably the NOAEL in females and 0.5 mg/kg, the NOAEL in

males; the significance of this finding is reinforced by other studies in rats, showing electrolyte changes to be associated with FK506 following oral administration. Histopathologic analysis revealed no changes attributable to dosing.

Comment: In this reviewer's opinion, the doses used in this study were too low for use in determining the 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 initiated; neither have been submitted to the IND; the second study which is being performed with higher doses than the first, is ongoing.

B13. Preliminary 2-week oral toxicity study of FK506 in dogs. Beagle dogs (ages 4-5 months; groups of 3, random sex) were given FK506 at doses of 0.32 or 1.0 mg/kg, or cyclosporine A at 10 mg/kg, daily for 2 weeks. While no dogs died, vomiting and bloody feces were seen from day 9 onwards in one dog receiving the high dose, correlating with intussusception of the colon. While this dog suffered gastrointestinal distress and other signs (hemato- and hepatotoxicity, immunotoxicity shown by atrophied lymphoid tissue, elevated BUN and electrolyte changes characteristic of kidney toxicity, and body weight decreases), other animals showed no severe signs of gastrointestinal toxicity with the exception of vomiting. The only effect on organ weights was a 15% decrease in thymus wt., seen at 1 mg/kg. Vomiting was also seen at 0.32 mg/kg in all 3 animals, mainly during the first 5 days. Given the frequency of vomiting, it would be unlikely that the full absorption of the doses was achieved.

GENOTOXICITY:

C1. Evaluation of FK506 in a chromosomal aberration test with chinese hamster lung cells line V79. Cells were incubated with FK506 at concentrations up to 100 ug/ml for 6, 24, or 48 h in the presence or absence of microsomal fraction S9; or with positive control agents, mitomycin C or dimethylnitrosamine (with activation). Concentrations of 100, 50, and 25 ug/ml were associated with 30-90% suppressions of the mitotic index, limiting the concentrations evaluated in the assay. No increase in chromosomal aberrations was noted in drug-treated plates up to concentration of 25 ug/ml, while significant increases were noted in positive controls.

C2. Evaluation of the potential of FK506 to induce unscheduled dna synthesis in the in vitro hepatocyte dna repair assay using the male F-344 rat. FK506 was dissolved in DMSO to yield concentrated solutions; these solution were added to hepatocyte cultures to achieved final concentrations ranging from _____ ug/ml with a maximum DMSO level of 1% v/v. Hepatocytes were isolated by harvesting the liver from 2 rats, digesting the livers with collagenase, and placing the cells in primary culture. Cultured cells were simultaneously exposed to FK506 and 10 uCi/ml of 3H-thymidine for 19 h. For a positive control, 0.5 ug/ml of 2-acetylaminofluorene (2-AAF) was used; %IR (cell in repair throughout liver) was considered negative if below 10%; the 2-AAF response was approximately 80%. While concentrations of 50 ug/ml of FK506 and higher were cytotoxic, %IR levels (in response to FK506 concentrations ranging from _____ ug/ml) ranged from _____ % and were scored negative in this assay. Thus, FK506 does not cause unscheduled DNA synthesis in rodent hepatocytes.

C3. FK506 effects on in vitro non-mammalian cell systems: I. Bacterial tester strains (TA, salmonella; WP, E. coli.) TA1535, WP2, and TA100 (base-pair mutations); TA1537 and TA 98 (frameshift mutations) were exposed (in the presence and absence of S9 microsomal preparation) to FK506 dissolved in DMSO in culture at 37 degrees for 16 h. Concentrations tested were _____ ug/plate; 2-aminoanthracene at _____ ug/plate, sodium azide at _____ ug/plate, 9-aminoacridine at _____

ug/plate, and 2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide at _____ ug/plate were used as positive controls. All positive controls produced positive results (2-aminoanthracene required metabolic activation) while FK506 produced negative results at all concentrations tested, with or without metabolic activation.

C4. FK506 effects on in vitro non-mammalian cell systems: II. (Reversion or Ames tests): Bacterial tester strains (TA, salmonella; WP, E. coli.) TA1535, WP2, and TA100 (base-pair mutations); TA1537 and TA 98 (frameshift mutations) were exposed (in the presence and absence of S9 microsomal preparation) to FK506 dissolved in DMSO in culture at 37 degrees for 16 h. Concentrations tested were _____ ug/plate; 2-aminoanthracene at _____ ug/plate, sodium azide at _____ ug/plate, 9-aminoacridine at _____ ug/plate, and 2-(2-Furyl)-3-(5-nitro-2-furyl)-acrylamide at _____ ug/plate were used as positive controls. All positive controls produced positive results (2-aminoanthracene required metabolic activation) while FK506 produced negative results at all concentrations tested, with or without metabolic activation.

C5. Mutagenicity study of FK506- chromosomal aberration test with chinese hamster lung cells (V79) in culture. Chinese hamster lung cells were incubated in vitro with FK506 at concentrations of _____ ug/ml for 24 h (without activation) or _____ ug/ml for 6 h (with activation); these concentrations were chosen in a pretest to determine maximum concentrations which would not inhibit cell growth. Metabolic activation was achieved by addition to the culture media of S9 microsomal preparation. Positive controls, mitomycin C (0.04 ug/ml) and dimethylnitrosamine (1000 ug/ml) showed positive results (chromosomal breaks and chromatid exchanges) in this system. FK506 at any of the concentrations tested, did not induce an increased incidence of chromosomal structural abnormalities or polyploids.

Comment: Appendices with actual data results were missing and requested by phone on 7/14/93.

C6. Evaluation of FK506 in the chinese hamster ovary (CHO) cell/hypoxanthine-guanine phosphoribosyl transferase (HGPRT) gene mutation assay. Mutations in these cells are detected by resistance to 6-thioguanine in the growth media. Cells were incubated (with and without S9 microsomal fraction) with FK506 for 4 h at concentrations of _____ ug/ml; concentrations higher than approximately 30 ug/ml caused reductions in growth rates; 50 ug/ml reduced growth rates 50%. Ethylmethane sulfonate (EMS; 200 ug/ml) and 3-methylcholanthrene (3-MC; 5 ug/ml) were used as positive controls in the absence of activation and dimethylbenzanthracene (DMBA; 3 ug/ml) was used in the presence of activation. Positive results, as defined by a greater than 3-fold increase in the vehicle-induced (background) incidence of revertants, were obtained with the positive controls (approximately 15-fold), and with one experimental sample with 40 ug/plate (approximately 3-fold background; with activation). In the same experiment, a negative result was obtained at 50 ug/plate, and the positive result at 40 ug/plate was not replicated in a second experiment. FK506 was not mutagenic in the CHO/HGPRT mutagenesis assay at the concentrations tested.

C7. Mutagenicity study of FK506- micronucleus test in mice following single oral dosing. BDF1 male mice (5/group) were given a single oral dose of distilled water, oral vehicle, or FK506 at doses of 125, 250, or 500 mg/kg of FK506. Twenty four, 48, and 72 h post-dosing, mice were sacrificed, and bone marrow cells were examined for micronucleated, polychromatic erythrocytes. Mitomycin C (2 mg/kg) was used as a positive control and produced a 50-fold increase in the incidence of micronucleated cells. At the single doses tested, FK506 did not induce increased levels of micronucleated erythrocytes, and therefore was not considered clastogenic in this assay.

C8. Mutagenicity study of FK506- micronucleus test in male and female mice. A similar (to C7) study was performed in male and female mice (5/sex/group). The only dose of FK506 tested was 500 mg/kg. At the single doses tested, FK506 did not induce increased levels of micronucleated erythrocytes, and therefore was not considered clastogenic in this assay.

REPRODUCTIVE TOXICITY:

D1. Study of FK506 on fertility and general reproductive performance in rats (Segment I). CD rats (30/sex/group) were given oral vehicle (suspending agents in distilled water) or FK506 at doses of 0.32, 1, or 3.2 mg/kg (LD, MD, and HD, respectively). The clinical maintenance dose of FK506 is approximately 0.15 mg/kg (or 10 ng/ml, whole blood levels), equivalent on a body surface area basis (correction by a factor of 6.3) to a rat dose of approximately 1 mg/kg, the MD. Males were dosed for 60 days prior to mating, during mating (a maximum of 15 days), and up until day 13 of gestation, when they were sacrificed and necropsied. Females were dosed for 14 days prior to mating, during mating (a maximum of 15 days), during gestation (typically 20-21 days; dams not delivering by day 25 were sacrificed to determine cause), and during lactation (21 days). One-third of the dams were sacrificed and necropsied at day 13 of gestation; two-thirds were permitted to continue through parturition and nursing. Litters were culled to 10 pups each at day 4 of lactation to an equal number/sex where possible using a random number table. Clinical observations, indices of reproductive performance, estrous cycle observations, body weight, food consumption, and necropsy results of sires, dams, and pups were monitored during the study with the exception of food consumption during cohabitation and mating.

Four deaths occurred on study; a control animal died from gavage error; 3 animals died in the 3.2 mg/kg (HD) group. One male was sacrificed in extremis on day 76 following signs of emaciation, labored breathing, sensitivity to touch, circling, tremors, and leaning. Two females died, one on day 43 (lactation day 2) with no signs except blood in one uterine horn; the other on day 51 (day 10 of lactation), showed abnormal signs of emaciation, swollen ventral abdomen, enlarged spleen, mottled lungs, and abscess in one uterine horn. Clinical signs suggestive of neurological involvement and soft stools were evident in HD males; additionally, decreased body weight gains (BWG) and food consumption (FC) were noted. In the HD groups, lower body weight gains were seen during gestation, but this was attributable to the low total litter weights. Evidence of maternal toxicity was decreased body weights at lactation day 7 in the HD only; also HD dams showed reduced food consumption during gestational intervals 0-7 and 7-13 and during lactation. Maternal toxicity was not evident at the MD. **No effects of drug were seen on production or release of corpora lutea or conception rate; however, effects on other aspects of female reproduction were marked at the HD.** These effects were lengthened diestrus, increased copulatory interval [average time until confirmed mating, and increased pre- and post implantation losses (early resorptions only)]. These effects resulted in a 40% and 79% reductions in live litter size at the gestation day 13 and postpartum, respectively. Increased preimplantation loss was also evident at the MD. Preimplantation losses for the 4 groups were: control, 5 lost from 2 dams; LD, 9 lost from 4 dams; MD, 28 lost from 7 dams; and HD, 27 lost from 6 dams. Although this effect was evident at a low incidence in the controls, the site and dam incidence was 5-fold and 3.5-fold the control incidence, respectively, at the MD level. The dose-trend and litter distribution suggest the biological significance of this effect. Marked effects on parturition were observed including increased average time to delivery among HD dams (23 vs. 21 days); 75% of the HD dams failed to deliver spontaneously; 4 dams displayed partial deliveries with retained pups found to be nonviable in utero at terminal necropsy (7 pups from 4 dams); 3 MD dams retained pups (4 pups from 2 dams) or placenta (1 placenta, 1 dam); 1 LD dam retained 1 pup; and no retentions were evident in control animals. The dose dependence and litter distribution of this effect suggest drug-related abnormalities in parturition in the MD group.

While the pups delivered of the HD dams were normal in weight, viability was significantly reduced, with only 30% of the pups surviving to lactation day 7; pups surviving to day 7 also survived to day 21. Mean weights were reduced in this group at day 7, but subsequent weighings showed that between days 7 and 14, mean weights increased to control levels and remained equal until day 21. As reported by the sponsor (no data table included; requested 7/2) there were no consistent skeletal or visceral abnormalities in the pups which died; however, 1 pup from each of 2 dams were noted to have an unusual intraventricular septal defect. (An additional dam was seen to have a pup retained in utero which had ablepharia, cleft palate, and craniorachischisis.) Among the MD pups which died, one had a vertebral malformation, another had a 7th rib, and another had hydronephrosis. (After 1 HD dam died, her litter was sacrificed; of these pups, 3 had hydronephrosis.)

Comment: Confirmation of the malformation data in the form of individual pup necropsy data has been requested on 7/2/93.

D2. Developmental toxicity study in rats of p.o. FK506 (Segment II). Female CD rats (40/group), designated the F0 generation, were given oral vehicle or FK506 at doses of 0.32, 1.0, or 3.2 mg/kg from gestation days 7-17 (during F1 organogenesis). On gestation day 20, half of the F0 females were subjected to cesarian section and necropsy; 10 of the remaining 20 were permitted to deliver. From those 10 litters, effects on the F1 litter viability were enumerated; culling of the F1 pups was performed on lactation day 4. Following lactation day 21, the F1 pups were further selected (2/sex/litter) for testing developmental and behavioral parameters (1/sex/litter), as well as for evaluating fertility and reproductive parameters (1/sex/litter; at aged 90 days) and effects on the F2 generation. The following behavioral and developmental indices were tested in the F1 rats: static righting reflex, pinna detachment, cliff aversion, eye opening, air-drop righting reflex, neuropharmacological evaluation (Irwin test), auditory response, rotarod performance, activity and emotionality (Digiscan® activity monitor), and passive avoidance test. The latter two tests are known to be sensitive behavioral indicators of adverse effects on neurological development. No effects of F0 dosing were seen on the behavior, development, or reproductive function of the F1 generation, nor were any effects seen on the viability, weight, or clinical appearance of the F2 pups. Maternally toxic effects were seen in the HD F0 dams; these effects were reduced body weight gain (41%) during gestation days 13-18 and reduced food consumption (23%) during days 7-14. Two mortalities which may have been related to drug treatment (exact cause undetermined) were both in the HD group and occurred on days 13 and 23 of gestation; the former was associated with dilated kidney and blood in the urine; the latter was associated with parturition.

Effects on the F1 generation included decreased fetal body weights in the MD and HD groups, and was consistent with a slightly increased incidence of developmental variations (101 HD pups rather than 67 in controls showing unossified bones and rib variations). A slight (not statistically significant) increase in post-implantation loss was seen in the HD only which correlated with a slight increase in late resorptions (0.2/dam rather than 0/dam in controls) and decrease in live litter size at birth (a 20% reduction in the number born live/number of implantations; effect not evident at cesarian section). Decreased pup viability was also seen on days 0 and 1 in the HD group; pups living to day 4 were still alive at day 21. Of the pups found dead on these days (8), 2 were cannabilized, 1 had an interventricular septal defect (seen also in the previous study). No observations seen in pups found dead were seen in more than 1 pup; no effects appeared to be dose-responsive.

D3. Perinatal and lactation study of FK506 in rats (Segment III). Female Crl:CD VAF/Plus rats, designated the F0 generation, were given oral vehicle or FK506 at doses of 0.32, 1.0, or 3.2

mg/kg (LD, 25 mated; MD, 31 mated; and HD, 33 mated; respectively) from gestation day 17 through parturition (on gestation day 21 or lactation day 0) through lactation day 21, when the F1 generation was weaned; culling of the F1 pups was performed on lactation day 4. The F1 was only exposed in utero for 5 days after organogenesis, and are likely to have been exposed to FK506 or its metabolites during nursing as shown in the section below on pharmacokinetics. The F1 pups were selected (2/sex/litter) for testing developmental and behavioral parameters (1/sex/litter), as well as for evaluating fertility and reproductive parameters (1/sex/litter; at aged 90 days) and effects on the F2 generation. The following behavioral and developmental indices were tested in the F1 rats: static righting reflex, pinna detachment, generalized hair growth, tooth eruption, cliff aversion, eye opening, corneal reflex, pupillary reflex, air-drop righting reflex, neuropharmacological evaluation (Irwin test), pain response, testicular descent, auditory response, vaginal opening, rotarod performance, activity and emotionality (Digiscan® activity monitor), and passive avoidance test. The latter two tests are known to be sensitive behavioral indicators of adverse effects on neurological development. The only effects seen in the behavioral and developmental tests was slight delays in eye opening and testicular descent. No mortalities or changes in normal clinical signs were detected on study.

Maternal toxicity was shown by decreased body weight gain (BWG; 72%) and food consumption (FC; 40%) in the HD F0 dams during gestation days 17, 18, 19, and 20, the period during which dosing commenced. Body weight gains were increased (>2x) in the HD dams during lactation days 0-21; during day 0-7, all groups gained similarly; during days 7-14, the HD group gained 40% more than the other groups; and during days 14-21 when control, LD, and MD dams lost 14-22 g of weight, HD dams gained 8 g. These effects on BWG during lactation are paradoxical when compared with FC patterns; FC among HD dams was reduced significantly during lactation days 0-7 (30%) and days 7-14 (17%).

Only one dam on study did not deliver and was confirmed gravid; a HD dam. The results of the Segment I study showed a marked adverse effect of 3.2 mg/kg FK506 on the parturition index. The birth index (the number of live newborns per no. of implantations) was not reduced in this study; however the HD F1 pups showed reduced viability between lactation days 0 and 4 (78% of the number born viable remained at day 4 in the HD, vs. 95-97% in the other groups). Body weights of the HD F1 pups were lower than weights of control pups during lactation (approximately 20%) and maturation (10% lower, out to 20 weeks). Body weights of the MD F1 animals was also reduced (approximately 10%) to a degree which was statistically significant by lactation day 7. Among the F1 pups which died, 48 pups from 10 HD litters were examined for gross abnormalities; 6/48 showed kidney hydronephrosis but no other abnormalities were evident. Following maturation of the F1 generation, young adults were mated; no effects on the F1 reproductive capacities or the F2 pups were noted.

Comment: 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. This effect was evident only during the later stages of lactation, indicating that FK506 may inhibit lactation.

D4. Segment II reproductive toxicity study in New Zealand white rabbits. Pregnant does were dosed orally with vehicle or FK506 at doses of 0.1, 0.32, or 1.0 mg/kg during organogenesis of the

fetus, day 6-18 in the rabbit. Parturition normally occurs at day 30 in this species; on day 29, all dams were sacrificed and full examination was made of reproductive organs and fetuses. Maternal toxicity was evident in the LD, MD, and HD groups for the day 6-18 time period as evidenced by a 50% decrease in body weight gain in the LD group, no BWG in the MD group, and a 154 g (approximately 5%) weight loss in the HD group. Decreased food consumption was also seen in the MD (10%) and HD (50%) groups during the dosed interval. Clinical signs were also seen in the MD and HD group including reduced stool, soft stool, increased lacrimation, and stained fur (inadequate grooming). Two dams aborted; one on day 29 and the other on day 23 in the MD and HD groups, respectively. Adverse effects on reproduction and fetal health were evident in the MD and HD groups. At the HD, a 4-fold increase (over control level) was seen in post-implantation loss, which corresponded with decreased litter sizes. Also seen in the HD fetuses was an increased incidence of developmental variations (over control levels; unossified sternabrae, fetal and litter incidence increased 50%; greater than 12 pairs of full ribs, fetal incidence increased 2-fold; increased 27 presacral vertebrae, 2-3 fold increases in fetal and litter incidence) and malformations (cardiovascular: ventricular hypoplasia, interventricular septal defect, bulbous aortic arch and stenosis of arch and ductus arteriosus; omphalocele, interrupted ossification of vertebral arch, vertebral malformation, rib malformation, and gallbladder agenesis). The total number of HD malformations was 22 out of 12 fetuses, from 8 does; litters from a total of 16 does were examined in the HD group. The rates of malformations in the MD groups was similar to control; 2 malformations in 2 fetuses, from 2 litters out of 18 does. While the vertebral malformation appeared once at the MD, twice at the HD (2%), and not at all in the LD and control groups, and appeared to be dose-responsive, this is a common malformation seen in historical controls at a rate of 0.6%; therefore, in this reviewer's opinion, increased rates of malformation are not seen at the MD. (A similar case is true for the increased 27th presacral vertebrae and unossified sternabrae; slightly increased rates of variations or trends upward were evident at the MD, but only for the most common variations.)

INTRAVENOUS VEHICLE TOXICITY:

E1. Reactions to polyoxyethylene hydrogenated castor oil 60 (HCO-60). Reactions to the the formulating agent, HCO-60 (diluted with saline), were evaluated in several species. Dogs (3/group) were given i.v. injections of 0.625, 1.25, 2.5, or 10 mg/kg; one dog given 10 mg/kg under anesthesia was bled and liver and skin section from the ear, abdomen and lip were fixed in Carnoy's and stained with toluidine blue and examined to determine mast cell degranulation patterns. In a separate group, an i.v. injection of 10 mg/kg was administered immediately following an injection of 5 mg/kg of diphenhydramine or cimetidine; blood pressure, and clinical signs were monitored. Dog blood was also incubated in vitro with 0.01, 0.1 or 1.0 mg/ml of HCO-60 for 15 min to determine histamine release into the supernatant. Monkeys (3/group) and rabbits (5/group) were given i.v. injections of 50 or 100 mg/kg; guinea pigs (5/group), and rats (5/group) were given i.v. injections of 10 or 100 mg/kg. Plasma histamine was monitored 10, 30, and 60 min following injection. Doses of 1.25 mg/kg and higher were associated with decreased spontaneous motility and blood pressure (40% drop 10 min following injection of 10 mg/kg), as well as elevated plasma histamine (increase to 600 ng/ml 10 min following injection of 10 mg/kg; 200 ng/ml, after 2.5 mg/kg), flush, swelling, and itching in dogs. No such changes were evident in monkeys, rabbits, guinea pigs, or rats. Mast cells in the skin were degranulated; those in the liver were not. No direct effect of HCO-60 on blood was noted. The pretreatment with diphenhydramine (not cimetidine) helped alleviate the effects of HCO-60 on blood pressure and spontaneous motility, but flushing and swelling was still evident.

E2. Single dose toxicity study of HCO-60 in rats by i.v. dosing. Jcl:SD (Sprague-Dawley) rats (5/sex/group) were given a single i.v. dose of HCO-60 at 1000 or 2000 mg/kg. Rats were observed for 14 days. No abnormal signs or effects on body weight gain were observed.

E3. HCO-60 toxicity to rats by repeated i.v. administration for 4 weeks Crl:CD SDBR rats (12 males, 12 females) were given i.v. injections of HCO-60 (400 mg/ml in ethanol, diluted 1:50 with saline) at doses of 40 mg/kg daily for 28 days. No effects were seen on body weight, food consumption, water consumption, ophthalmoscopic, hematological, serum chemical, urine analytical, organ weight, or histopathological parameters compared to historical controls from this strain and age of rats; no control data were supplied with the study report.

E4. Four week i.v. toxicity study of HCO-60 in rats. Sprague-Dawley rats (10/sex/group) were given i.v. doses of saline or 100, 320, or 1000 mg/kg of HCO-60, the i.v. vehicle used in the clinical i.v. formulation of FK506. The HCO-60 is an oily solid at room temperature, and must be warmed slightly to form a liquid which can be diluted with saline solution. Injection volumes were adjusted with saline to equal 5 ml/kg (a 1.25 ml injection volume in a 250 g rat). At 100 mg/kg, spleen weight increased (males), but was not above the normal range. At 320 mg/kg and higher, glucose (females) and liver wt. increased; AP and cholesterol decreased slightly. Vacuolation was seen in macrophages of the femoral bone marrow, spleen, lymph nodes, adrenal, testis, ovary, and uterus. Vacuolation of epithelial cells of the proximal tubules of the kidney were seen (in the absence of any change in BUN, creatinine, or serum electrolytes). At 1000 mg/kg, triglycerides, WBC, LYM, platelets, and segmented neutrophils (males and females) decreased; hematocrit, RBC, and HG, ovary weight and corpora lutea (females) decreased; and kidney wt. (males and females) increased. The NOAEL in this study was 100 mg/kg.

E5. Mutagenicity of HCO-60 (micronucleus test) in BDF1 mice. Mice (5/sex/group) were given HCO-60 at a single i.p. dose of 2000 mg/kg and mice were sacrificed 24, 48, or 72 h later to evaluate the bone marrow for the incidence of micronucleated polychromatic erythrocytes. Positive results were obtained for the control, an i.p. dose of 2 mg/kg of mitomycin C. No difference was evident in results from saline or HCO-60 treated mice, therefore, this compound is considered negative in this assay of clastogenic potential.

E6. Evaluation of HCO-60 in a chromosomal aberration test with chinese hamster lung cells line V79. Cells were incubated in medium containing the test article for 6 h (followed by refeeding for 18 h), 24, or 48 h. Concentrations of 5000, 2500, or 1250 ug/ml were tested for these times, respectively in the presence and absence of S9 activation. Chromosomal aberrations were not evident in any group with the exception of the positive control groups, exposed to mitomycin C (0.04 ug/ml) or dimethylnitrosamine (5000 ug/ml, used with activation); therefore, this compound was not considered positive for clastogenicity in this assay.

E7. Antigenicity study of HCO-60 and cremophor in guinea pigs. ASA and PCA were performed using guinea pigs, as explained in experiment F4, to evaluate sensitization and IgE development to the HCO-60 used in the i.v. formulation of FK506. For ASA, the i.v. dose of HCO-60 used was 100 mg/kg; for PCA, 20 mg/kg was used s.c. The positive control in this assay was egg albumin (EA) at a dose of 5 mg/kg. Three animals/group were used for testing sensitizing guinea pigs for ASA, involving administration of the test substance with or without Freund's adjuvant. One out of 3 animals which was not previously sensitized, died following injection of HCO-60. For the 10 animals sensitized with HCO-60, no animals died following rechallenge, which should have confirmed the finding of mortality if the effect were reproducible. Therefore, the one death is considered to be unrelated to the test agent. All animals sensitized and rechallenged with EA died.

E8. Correlation between plasma histamine levels and infusion time of HCO-60 in dogs. Plasma histamine levels were measured following the infusion of 4 mg/kg of HCO-60 in beagle dogs; infusion times were either 30, 60, or 120 min. Plasma histamine levels during each infusion were a maximum of 84, 31, or 6 ng/ml, respectively. Therefore, the histamine release known to occur in dogs in association with HCO-60 i.v. administration may be controllable by the adjusting the rate of infusion.

E9. HCO-60 toxicity to baboons by repeated i.v. administration for 4 weeks. HCO-60 was administered i.v. to 3 male and 3 female baboons at a dose of 40 mg/kg, daily for 28 days. No

control group was evaluated; results were compared with historical controls and pretreatment values. No effects were seen on clinical observation, ophthalmoscopic, urinalysis, serum chemical, hematologic, electrocardiographic, histopathologic, or gross necropsy parameters.

SPECIAL TOXICITY:

- F1. Morphological and functional changes in islet of Langerhans in FK506 treated rats (non-GLP). Pancreatic islet cell vacuolation has been detected in subchronic toxicity studies of FK506 in rodents. To evaluate this effect further, a study was conducted with oral doses of 1, 5, or 10 mg/kg for 14 days in 6 wk-old male Sprague-Dawley rats (12/group). Islets of Langerhans were evaluated histopathologically following dosing (7/12) and after a 2-wk recovery period (5/12). While higher plasma glucose levels were not evident under normal feeding condition, a glucose challenge (3 g/kg following 18 h fasting; days 15 and 22) revealed inadequate insulin response in the 10 mg/kg group and glucose intolerance in the 1, 5, and 10 mg/kg groups measured at 30 to 240 min following challenge. Vacuolation and degranulation was evident in the 5 and 10 mg/kg group, but these responses were reversible following 2 weeks of recovery.
- F2. Effects of FK506 and CyA on exocrine function of the rat pancreas. Carbachol-stimulated (1×10^{-6}) amylase and lipase secretion from the pancreas were unaffected by 14 days of oral dosing with 1 or 5 mg/kg of FK506 administered to 5-wk old male Sprague-Dawley rats. Pancreas was harvested from drug and vehicle-treated rats, incubated with carbachol for 10 min, homogenized, and supernatants were tested for amylase and lipase using a commercial kit. CyA (50 mg/kg) did affect exocrine secretion as shown by reduced amylase secretion induced by carbachol.
- F3. Antigenicity study of FK506 in mice. IgE antibody formation was evaluated in BDF1 mice following 15 injections of FK506 at a dose of 1 mg/kg (with or without 4 mg aluminum hydroxide/mouse as an adjuvant), spread over 19 days; egg albumin (EA; 1 ug/mouse) was used as a positive control. Ten days later, mice were bled and blood was tested using a PCA (passive cutaneous anaphylaxis) assay in the rat. A small aliquot of mouse serum was injected under the clipped skin of the rat. Serum samples from mice were injected i.v. into rats, along with the putative antigen, FK506, and Evans Blue dye. The serum dilution correlating with a blue tinting of the skin (in the area previously injected) of $\geq 5 \text{ mm}^2$ in diameter was used to characterize the potency of the response, correlating with the titre of the IgE antibodies formed in response to the antigen. No serum injections, with or without adjuvant, elicited a reaction in this assay with the exception of those from the positive control, which elicited a reaction when diluted 640-fold.
- F4. Antigenicity study of FK506 in guinea pigs. Skin reactivity, passive cutaneous and acute active systemic anaphylaxis were tested following 3 injections of FK506 at a dose of 5 mg/kg (once/week for 3 weeks in Slc:Hartley male guinea pigs); 2 days later, blood was withdrawn for PCA testing in a naive guinea pig. For the skin testing, [2,4,6]-trinitrobenzene sulfonate (TNBS) was used as a positive control agent, along with Freund's complete adjuvant. An immediate allergic reaction resulted from an i.d. injection of 50 ug of TNBS; 50 ug of FK506 was used for comparison in an evaluation of skin reactivity. For active anaphylactic responses in sensitized animals, TNBS was used to prepare an EA (egg albumin)-TNP (trinitrophenol) conjugate for use as a positive control. This agent was used at a dose of 2.5 mg/kg in an i.v. dose to elicit active systemic anaphylaxis (ASA). Passive cutaneous anaphylaxis (PCA) was performed as stated in the experiment described above, using EA-TNP as a positive control. None of these assays showed a positive anaphylactic response to, or sensitization caused by, FK506.
- F5. Local irritation test of FK506 injectable formulation in rabbits by i.m. injection. New Zealand White rabbits (3) were injected with one of 2 concentrations of FK506, either a 0.01 or 0.1% solution of FK506 in the i.v. vehicle (400 mg of HCO-60 and 0.6 ml ethanol, diluted 1:10 with saline solution). Positive controls used were either 0.425% or 17% acetic acid; reactions to the test articles were gauged relative to the damage caused to the local muscle by the acetic acid controls. Muscle samples were examined macroscopically either 2 or 14 days following a single

injection. Effects of either of the FK506 solutions were not different as gauged in this assay from the placebo. Injuries were seen only on day 2 for FK506 or placebo, and were characterized as hemorrhage and whitish coloration. Acetic acid at 0.425% caused similar reactions, but the effects persisted to 14 days.

METABOLISM AND PHARMACOKINETICS

STUDY SUMMARY:

1. Takada K, Oh-Hashi M, Yoshikawa H, Muranishi S, Nishiyama M, Yoshida H, Hata T, and Tanaka H. Determination of a novel potent immunosuppressant (FK506) in rat serum and lymph by high-performance liquid chromatography with chemiluminescence detection. *J Chromatog, Biomed Appl* 1990; **530**: 212-18.
2. Christians U, Kruse C, Kownatzki R, Schiebel HM, Schwitzer R, Sattler M, Schottmann R, Linck A, Almeida VMF, Braun F, and Sewing K. Fr. Measurement of FK506 by HPLC and isolation and characterization of its metabolites. *Transplant Proc* 1991; **23**: 940-1.
3. Iwasaki K, and Matsuoka H. Pharmacokinetic parameters of ¹⁴C-FK506 in the rat. Fujisawa Pharmaceutical Company Report CRR 920227, 1992.
4. Iwasaki K, Nagase K, and Noda K. Blood concentrations of FK506 in the rat: dose proportionality study. Fujisawa Pharmaceutical Company Report CRR900064, 1990.
5. Iwasaki K, Shiraga T, Nagase K, Hirano K, Nozaki K, and Noda K. Pharmacokinetic study of FK506 in the rat. *Transplant Proc* 1991; **23**: 2757-9.
6. Iwasaki K, and Nagase K. Pharmacokinetics of FR900506 (FK506) in rats during repeated dosing. Fujisawa Pharmaceutical Company Report CRR880352, 1988.
7. Kagayama A, Fujisaki J, Kaibara A, Tanimoto S, and Hata T. Blood concentrations of FK506 in rats-gastrointestinal absorption site of FK506. Fujisawa Pharmaceutical Company Report CRR920074, 1992.
8. Kagayama A, Tanimoto S, Tanaka Y, and Hata T. Blood concentrations of FK506 in rats - Effect of feeding on oral absorption of FK506. Fujisawa Pharmaceutical Company Report CRR920073, 1992.
9. Honbo T, Kobayashi M, Hano K, Hata T, and Ueda Y. The oral dosing form of FK506. *Transplant Proc* 1987; **19**: 17-22.
10. Iwasaki K, Nagase K, and Noda K. Plasma protein binding of FK506. Fujisawa Pharmaceutical Company Report CRR900042, 1990.
11. Iwasaki K, Nagase K, and Noda K. Distribution of FK506 in the blood. Fujisawa Pharmaceutical Company Report CRR900049, 1990.
12. Iwasaki K, Nozaki K, and Noda K. Tissue distribution of radioactivity in rats dosed with FK506. Fujisawa Pharmaceutical Company Report CRR920224, 1992.
13. Fujiwara T, Tozuka Z, and Tokuma Y. Distribution of radioactivity in rats dosed with ¹⁴C-FK506 observed by whole-body autoradiography. Fujisawa Pharmaceutical Company Report CRR910449, 1991.
14. Esumi Y, Jin Y, Katoh Y, Miyake T, Takahashi M, and Shiira M. Transfer of radioactivity to fetus and milk after oral dosing of ¹⁴C-FK506 observed by whole-body autoradiography. Fujisawa Pharmaceutical Company Report CRR910449, 1991.
15. Iwasaki K, Shiraga T, Matsuda H, Noda K, Yamazoe Y, Nagata K, Yasumori T, Ozawa S, Shimada M, Murayama N, and Kato R. Effect of repeated oral doses of novel immunosuppressive macrolide lactones in hepatic mixed-function oxidase system in the rat, comparative study with cyclosporin. *Arzneim-Forsch/Drug Res* 1992; **42**: 340-4.
16. Yoshimura R, Yoshimura N, Kusunose E, Hamashima T, Ohsaka Y, Kusunose M, Oka T, Kishimoto T, and Maekawa M. A comparative study between cyclosporine and FK506 effects on renal microsomal P450. *Transplant Proc* 1991; **23**: 311-3.
17. Vincent SH, Karanam BV, Painter SK and Chiu S-HL. *In vitro* metabolism of FK506 in rat, rabbit and human liver microsomes: Identification of a major metabolite and of cytochrome P450 3A as the major enzyme responsible for its metabolism. *Arch Biochem Biophys* 1992; **294**: 454-60.
18. Iwasekaki KA, Nagase K, and Matsuda H. Metabolism of ¹⁴C-FK506 in rat and human liver microsomes. Fujisawa Pharmaceutical Company Report CRR920238, 1992.
19. Shiraga T, Shimataki K, Sato A, Fujioka M, and Iwasaki K. Isolation and identification of FR900506 metabolites produced by rat hepatic microsomes. Fujisawa Pharmaceutical Company Report CRR920276, 1992.
20. Nagase K, Yoshida H, and Iwasaki K. First pass effect of FK506 in rats. Fujisawa Pharmaceutical Company Report CRR920228, 1992.
21. Effect of 23 drugs of metabolism of FK506 by human liver microsomes
22. Enterohaptic circulation of radioactivity in rat after p.o. administration of [¹⁴C]FK506.
23. Iwasaki K. Absorption and excretion of ¹⁴C-labelled FR900506 in rats. Fujisawa Pharmaceutical Company Report CRR880349, 1988.

24. Excretion profile of radioactivity after a single and repeated oral dose of [¹⁴C]FK506 to male rats.
25. Pharmacokinetics of FK506 in the baboon.
26. Pharmacokinetics of plasma FK506 in baboons during repeated dosing.
27. The distribution and excretion of FK506 in baboons after single i.v. doses of 1 and 10 mg/kg.
28. Absorption, distribution and excretion studies of FK506 in baboons.

Review:

Determination of a novel potent immunosuppressant (FK506) in rat serum and lymph by high-performance liquid chromatography with chemiluminescence detection. (see ref. 1) An HPLC method was developed which was used for determining FK506 levels in rat plasma and lymph by prelabelling with dansyl hydrazine, and detection using chemiluminescence; detection sensitivity was 0.5 ng/ml. The more widely used assay, an enzyme immunoassay technique is less specific because the antibody used cross reacts with some metabolites of FK506.

Measurement of FK506 by HPLC and isolation and characterization of its metabolites. (see ref 2) FK506 was incubated ex vivo using human liver microsomes and metabolites were evaluated using HPLC and fast atom bombardment mass spectrometry (FAB-MS). Metabolites were also evaluated for immunosuppressive activity by inclusion in an assay of mitogen (phytohemagglutinin; PHA) stimulated lymphocyte proliferation. Nine metabolites were identified by this method. Metabolic pathways were typical of Phase I metabolism - products were mainly o-demethylated and hydroxylated. Demethylated and double-demethylated metabolite fractions were shown to be inhibitory of lymphocyte proliferation and possessed IC₅₀s of approximately 10 ug/l; the IC₅₀ of FK506 in this system was approximately 1 ug/L. These two metabolites were also generated by rat small intestine microsomes.

Pharmacokinetics: (see refs. 3-5) Based on single dose studies in rats, both FK506 and total radioactivity are more completely detected by measuring whole blood than by measuring plasma levels; a point also illustrated by the 20% smaller Vd seen when measuring whole blood. FK506 is rapidly metabolized; exposure to metabolites is 3-fold the exposure to FK506 as shown by the higher radioactive AUC, which is not accounted for by radioactivity due to FK506 itself. The clearance of these metabolites is nearly 2X slower than the clearance of FK506. The T_{max} of FK506 following oral treatment is approximately 0.5 h and the t_{1/2} of FK506 is approximately 6 h; these parameters were not related to dose. C_{max} and AUC for FK506 are related to dose (roughly proportional) in the dose range of 1.0-10.0 mg/kg. The bioavailability of FK506 from the oral route is best measured in whole blood and is approximately 12% based on equal nominal doses administered orally and intravenously. Results are summarized in the following table.