Methods:

Each of the ten animals used received applications of FR900506 ointment at three different concentrations, as well as both a positive (4-HA/TRA) and negative control (placebo ointment) application. Five test sites measuring ~12.5 cm^2 each were designated on each side of the dorsal midline of each animal. Sites were identified by side (L or R) and numbered (1-5, starting from the head). The hair on each test site was clipped on an as needed basis.

FR900506 ointment, 4-HA/TRA and placebo ointment were applied unoccluded twice daily (~5-6 hours apart) for 8 weeks. FR900506 ointment or placebo ointment was applied evenly to the test site by syringe at a dose volume of 0.1 ml/site. Prior to application of the second daily dose, the test sites were gently wiped off using water moistened cloth and blotted dry. The positive control (4-HA/RTA) was applied evenly to the test site at a volume of 0.025 ml/site.

Dosing:

- *species/strain:* male New Yucatan miniature pigs
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 4-6 months
- *weight:* 19.2-27.8 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* N/A
- *route, form, volume, and infusion rate:* route = topical

Site Allocation Table

Left Side		Right Side	
Site Number	Application	Site Number	Application
1	Placebo Ointment	1	Placebo Ointment
2	0.03% FR900506 Ointment	2	0.03% FR900506 Ointment
3	0.1% FR900506 Ointment	3	0.1% FR900506 Ointment
4	0.3% FR900506 Ointment	4	0.3% FR900506 Ointment
5	4-HA/TRA	5	4-HA/TRA

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 618147K
0.03% FR900506 ointment – lot # 618247K
0.1% FR900506 ointment – lot # 618547K
0.3% FR900506 ointment – lot# 618847K
2% 4-hydroxyanisole (4-HA) – Lot # 06418AN
0.01% all-trans-retinoic acid (TRA) – lot # 15822JG

Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously. The positive control solutions, 2% 4-HA and 0.01% TRA, were prepared weekly in a hydroethanolic solution.

Observations and times:

- *Mortality:* daily
- *Local dermal signs:* Test sites were examined weekly for signs of local irritation and hypopigmentation. Local irritation was graded using the Draize method. Hypopigmentation was graded on a 4 point scale. Grade 1 – Complete depigmentation, Grade 2 – Moderate and uniform hypopigmentation, Grade 3 – Small spots of hypopigmentation and Grade 4 – Normal skin color.
- *Body Weight:* weekly
- *Histopathology:* All administration sites were fixed in formalin. A representative section of each test site was stained with Fontana-Masson silver stain and examined microscopically for assessment of melanocyte pigment.

Results:

- **Mortality** No mortality was noted in this study.

- **Local dermal signs**

No treatment related signs of dermal irritation were noted in FR900506 ointment or placebo ointment treated animals. Positive control animals (4-HA/TRA) showed signs of mild irritation during week 8.

No treatment related effects on the cutaneous pigmentation of Yucatan miniature swine were noted in FR900506 ointment or placebo ointment treated animals. The mean hypopigmentation grades of all placebo ointment and FR900506 ointment treated sites were 4.0 during week 9.

Topical administration of the positive control (4-HA/TRA) caused induction of gross hypopigmentation in the skin of Yucatan miniature swine in study weeks 4 – 9. The reduction in skin pigmentation gradually increased from weeks 4 – 9. The mean hypopigmentation grades for left and right sides of pigs were 3.2 and 3.3, respectively, in week 4. In week 9, the mean scores on the left and right sides were 2.0 and 1.9, respectively.

- **Body Weight** No treatment related effects on body weight were noted in this study.

- **Histopathology** No treatment related effects on cutaneous pigmentation after histopathological examination was noted for FR900506 ointment and placebo ointment treated animals. Focal depigmentation (11 sites) or diffuse reduction (9 sites) in the numbers of pigment granules was noted microscopically in positive control treated animals.

Key Study Findings:

Topical administration of 0.03%, 0.1% and 0.3% FR900506 ointment and placebo ointment had no effect on the skin pigmentation of Yucatan miniature swine after eight weeks of twice daily application. Topical administration of the positive control (4-HA-TRA) caused a significant induction of gross hypopigmentation in the skin of Yucatan miniature swine in weeks 5 through 9. Microscopic examination of the skin sites revealed findings that were consistent with the gross observations.

Special Toxicology Study #6:

Comparison of FK506 (Tacrolimus) and glucocorticoids ointment on dermal atrophogenicity in rats

Study Title:	Comparison of FK506 (Tacrolimus) and glucocorticoids ointment on dermal atrophogenicity in rats
Study No:	R97-0020-506-P2-E
Amendment #, Vol #:	000, 15
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study completion:	1/14/97
GLP compliance:	No
QA- Report:	Yes () No (X)

Methods:

Test ointment (0.2 grams) was applied for 6 hours daily to the shaven dorsolumbar skin (3 x 3 cm area) of rats for 21 consecutive days. Each animal was fitted with an Elizabethan collar prior to application of test ointment. After 6 hours the test substance was removed by washing with a wet cloth. The sham control group underwent the same type of procedure.

Treatment groups included: 0.3% FR900506 ointment, 0.05% clobetasol 17-propionate (CP) ointment, 0.12% bethamethasone 17-valerate (BV) ointment, 0.05% clobetasone 17-butyrate (CB) ointment and 0.5% prednisolone (Pr) ointment. The corticosteroid ointments have the following strength of efficacy ranking according to the sponsor: CP (strongest), BV (strong), CB (medium) and Pr (weak).

Dosing:

- *species/strain:* male Jcl:SD rats
- *#/sex/group or time point:* 7 male rats/group
- *age:* 4 weeks
- *weight:* 105-132 g
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* N/A
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: No information was supplied in the study report.

Formulation/vehicle: No information was supplied in the study report.

Observations and times:

- *Body Weight:* weekly
- *Skin Thickness:* Prior to first dosing and then weekly. Skin thickness was measured according to the double skin-fold thickness method by a dial thickness gauge
- *Skin Biopsy:* The day after the conclusion of ointment application, the dorsal skin was removed, disc punched (3 cm in diameter) and weighed. The skin discs were fixed in formalin for H&E staining in one section and PCNA immunostaining (used as an index of epidermal proliferation) in another section.

Results:

- **Body Weight** Body weight gain was slightly decreased in FR900506, CP, CB and Pr ointment treated animals compared to sham control animals over the three week treatment period. Body weight gain was markedly decreased in the CP ointment treated animals compared to sham control animals. The body weight in the CP ointment group was only about 50% of the sham control animals body weight at the conclusion of the three week treatment period.

- **Skin Thickness**

The results of the various ointment treatments on skin thickness are summarized in the following table.

Treatment	Predosing Skin Thickness (mm)	1 Week Skin Thickness (mm)	2 Week Skin Thickness (mm)	3 Week Skin Thickness (mm)
Sham Control	1.71 ± 0.11	2.73 ± 0.26	2.97 ± 0.21	2.58 ± 0.15
0.3% FR900506 Ointment	1.72 ± 0.16	2.75 ± 0.25 (100.7)†	2.88 ± 0.19 (97.0)	2.39 ± 0.13* (92.6)
0.05% Clobetasol Propionate	1.71 ± 0.13	1.50 ± 0.09** (54.9)	1.23 ± 0.11** (41.4)	1.10 ± 0.07** (42.6)
0.12% Betamethasone Valerate	1.71 ± 0.06	2.56 ± 0.15 (93.8)	2.48 ± 0.18** (83.5)	2.20 ± 0.12** (85.3)
0.05% Clobetasone Butyrate	1.71 ± 0.08	2.31 ± 0.14** (84.6)	2.17 ± 0.13** (73.1)	1.75 ± 0.11** (67.8)
0.05% Prednisolone	1.72 ± 0.16	1.99 ± 0.24** (72.9)	1.90 ± 0.23** (64.0)	1.63 ± 0.15** (63.2)

Values are expressed as mean ± S.D. (N=6-7).
† - Values in ( ) are relative % thickness compared to sham control.
* - $p<0.05$, ** - $p<0.01$; Statistically significant difference from sham control.

The thickness of the skin treated with FR900506 ointment was similar to that observed in the sham control group for the week 1 and week 2 measurements. There was a slight but significant decrease in skin thickness for FR900506 ointment treated animals at the week 3 measurement. The skin thickness measurements were significantly decreased in the CP, CB and Pr treated groups starting at the week 1 measurements and in the BV treated group starting at the week 2 measurement. The CP treated group exhibited the strongest atrophogenic response after test article administration.

- **Skin Biopsy**

The results of the skin weight measurements obtained at the end of the study paralleled the skin thickness results reported above. No significant differences were observed in the skin weight between the FR900506 ointment treated animals and the sham control. There was a significant decrease in skin weight observed in all of the glucocorticoid ointment treatment groups compared with the sham control with the CP group showing the largest decrease and the BV group showing the smallest decrease.

The skin treated with FR900506 ointment was histopathologically similar to that in the sham control group. All of the glucocorticoid ointments decreased the subcutaneous fatty tissues, and CP, CB and Pr ointments reduced the thickness of the epidermis and dermis. There was atrophy of the sebaceous glands along with thinning of the subcutaneous muscular layers in rats treated with the CP ointment.

PCNA positive nuclei were identified in epidermal and follicular basal epithelial cells of the FR900506 ointment treated rats with a similar frequency to that in the sham control group. A small number of PCNA positive cells were detected in the skin of the BV treated rats, but none were seen in the CP, CB or Pr treated rats.

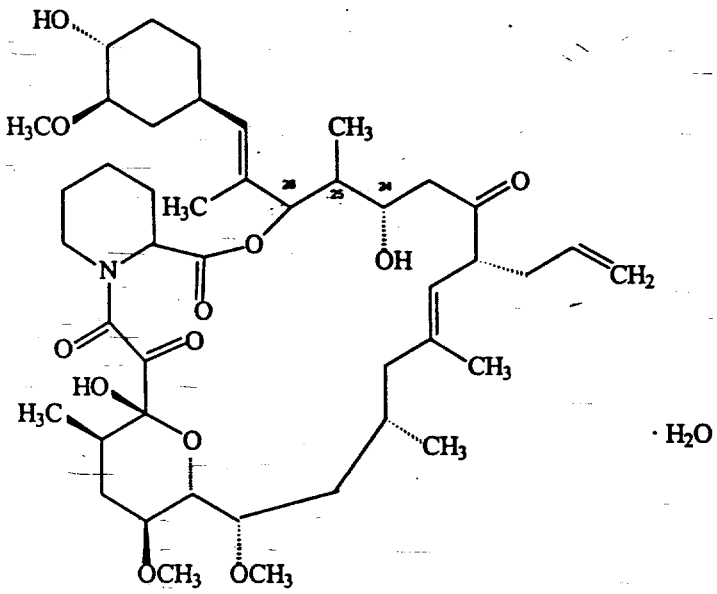
Key Study Findings:

The results from this experiment indicate that 0.3% FR900506 ointment did not induce skin atrophy after 3 weeks of daily treatment in rats. The glucocorticoid ointments induced skin atrophy with the following potency levels in descending order: CP>Pr>CB>BV.

Degradation Product Toxicology Studies (Submitted to the NDA and/or to IND ——

Background/Introduction:

The sponsor identified an impurity in the tacrolimus ointment as —— in Serial #50 in IND —— and stated that the level of this impurity was —— The —— impurity has been characterized as —— in which the —— from —— (refer to figure below).



FR900506

The sponsor has conducted five nonclinical toxicology studies for qualification of the —— impurity. Four of the studies were submitted to IND —— in Serial # 051 and have been reviewed previously. The 4 week repeat dose study protocol was submitted to IND —— Serial # 083 and reviewed previously. The final study report for the 4 week repeat dose study protocol was submitted to the NDA.

Degradation Product Toxicology Study #1:

Single dose toxicity of degradation compound —— of FR900506 ointment in mice

Study Title:	Single dose toxicity of degradation compound —— of FR900506 ointment in mice
Study No:	R96-0134-506-P2-E; GLR960362
Amendment #, Vol #:	000, 40
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	9/20/96
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Mice received a single intravenous injection of —— solution and toxicity was assessed for 14 days. The dose selection for this study was based on the results of a previous single dose toxicity study of FR900506 performed in mice. In the previous toxicity study using FR900506, all the animals died in the 100 mg/kg dose group and none of the animals died in the 56 mg/kg dose group. Therefore, the sponsor selected the 56 and 100 mg/kg doses of —— in the current study to compare the toxicity with that observed for FR900506.

Dosing:

- *species/strain:* Jcl:ICR mice
- *#/sex/group or time point:* 5/sex/group
- *age:* 6-7 weeks
- *weight:* 36.2 - 40.8 g for males and 29.2 - 32.4 g for females
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 56 mg/kg —— soln.) and 100 mg/kg (—— soln.)
- *route, form, volume, and infusion rate:* route = intravenous, vehicle = Polyethylene glycol 400 NF, volume = 2.5 ml/kg

Drug, lot#, radiolabel, and % purity: —— substance – lot # 722066L

Formulation/vehicle: ——was dissolved in Polyethylene glycol 400 NF

Observations and times:

- *Clinical signs:* twice daily for 14 days
- *Body weights:* days 1, 2, 4, 7, 10 and 14
- *Gross pathology:* at sacrifice

Results:

- **Clinical signs** Two of the male mice and all of the female mice died within 5 minutes after dose administration in the 100 mg/kg dose group. None of the animals died in the 56 mg/kg dose group. The clinical signs observed for the 100 mg/kg treated animals included clonic convulsion in 1 male and all the females immediately after dose administration. All of these animals died within 5 minutes.

- **Body weights** Slight and transient decreases in body weight were observed in the surviving animals of all the groups after dose administration. Thereafter, body weight increased normally throughout the observation period.

- **Gross pathology** No treatment related gross pathology effects were noted in this study.

Key Study Findings:

The toxic findings and mortalities of —— in the present study were similar to those of a previous single dose study of FR900506 in mice given the same two doses (56 and 100 mg/kg). The sponsor stated that these results suggest that —— and FR900506 are similar in the quality and severity of their single dose toxicity. It is difficult to compare the results from two separate studies due to potential differences in strains of animals and different testing conditions. In addition, there were only two doses tested in this study with no control group for comparison. It would have been more useful to have conducted this study with a side by side comparison of the toxicity of —— and FR900506 incorporating a control, low, mid and high dose groups for each test article. However, the preliminary data from this experiment do suggest that the —— impurity and FR900506 do elicit similar toxicities at equivalent doses.

Degradation Product Toxicology Study #2:

Local irritation study of degradation compound —— of tacrolimus ointment in rabbits (Primary dermal irritation)

Study Title:	Local irritation study of degradation compound —— of tacrolimus ointment in rabbits (Primary dermal irritation)
Study No:	R96-0135-506-12-E; GLR960366
Amendment #, Vol #:	000, 40
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	11/96
GLP compliance:	No
QA- Report:	Yes () No (X)
Methods:	

The back of the animals were clipped and depilated longitudinally with a depilatory cream the day before application of the test article. This area was divided into 2 or 4 - 5 cm^2 sections of skin and the opposite ones were abraded by making epidermal incisions with an injection needle. Test article (0.5 g of each) was applied to the abraded or intact sites, covered with surgical gauze and wrapped with adhesive tape for 24 hours. The test article was removed after 24 hours.

Dosing:

- *species/strain:* Male New Zealand White Rabbits
- *#/sex/group or time point:* 5/sex/group
- *age:* 46 weeks
- *weight:* 3.68-5.44 kg
- *satellite groups used for toxicokinetics or recovery:* N/A
- *dosage groups in administered units:* 0.5 grams of either placebo ointment, —— ointment or 5% sodium lauryl sulfate
- *route, form, volume, and infusion rate:* route = topical

Drug, lot#, radiolabel, and % purity: [redacted] ointment – lot # 63636XK
Placebo ointment – lot # 63626XK,
Sodium lauryl sulfate – lot # M4T9070 [redacted]

Formulation/vehicle: Placebo and [redacted] ointments were same as clinical formulation, Sodium lauryl sulfate was dissolved in white vaseline

Observations and times:

- *Local dermal signs:* Erythema, eschar and edema were measured 24 and 72 hours after test article administration according to the Draize method.

Results:

- **Local dermal signs** No signs of erythema, eschar or edema were noted at the intact or abraded skin site at 24 or 72 hrs after application of either the placebo or [redacted] ointments. Well defined to severe erythema was observed on the intact and abraded sites of all animals and very slight to severe edema on the abraded site of 1 animal 24 hours after application of 5.0% sodium lauryl sulfate. Erythema and edema were still present 72 hours after application and eschar was observed in sodium lauryl sulfate treated animals. The mean primary skin irritation index of the 5% sodium lauryl sulfate solution was 5.3 and was classified as a moderate irritant.

Key Study Findings:

The [redacted] ointment was classified as a non-irritant in rabbits under the conditions of this study.

Degradation Product Toxicology Study #3:

Four-week percutaneous toxicity study of a main degradation compound , [redacted] , of FR900506 ointment in rats

Study Title:	Four-week percutaneous toxicity study of a main degradation compound [redacted] of FR900506 ointment in rats
Study No:	1998-1011-PJ-1; GLR980300
Amendment #, Vol #:	000, 40
Conducting laboratory:	Toxicology Research Laboratories, Fujisawa Pharmaceutical Co., Japan
Date of study initiation:	2/3/98
GLP compliance:	Yes
QA- Report:	Yes (X) No ()

Methods:

Approximately 24 hours before treatment commenced, hair was clipped from the dorsal region of each animal. The treatment area was 5 cm x 5 cm. The treatment site was intact skin. Hair clipping and Elizabethan collar fitting were carried out for sham control animals.

Each dose (2 gm/kg/day) was applied to the appropriate dermal test site as a thin uniform layer. The animals were then fitted with a plastic Elizabethan collar for a six hour period. At the end of this period, the collar was removed and the dermal application site was washed with warm water and blotted dry. The treatment site remained unoccluded through out the treatment period. Animals were treated once daily for 28 days.

Dosing:

- *species/strain:* Crj:CD (SD) rats
- *#/sex/group or time point:* Refer to dosing table below
- *age:* 9-10 weeks
- *weight:* 327-390 grams males; 220-250 grams females
- *satellite groups used for toxicokinetics or recovery:* Refer to dosing table below
- *dosage groups in administered units:* Refer to dosing table below
- *route, form, volume, and infusion rate:* route = topical, for additional information refer to table below

Dosing Table

Treatment	Dose (mg/kg/day)	Number of Main Study Animals	
		Males	Females
Sham Control	0	10	10
Placebo Control	0	10	10
1.0 % FR900506 ointment	20	10	10
[redacted] ointment	20	10	10

Drug, lot#, radiolabel, and % purity: Placebo ointment – lot# 603482K
[redacted] ointment – lot# 603682K
1.0% FR900506 ointment – lot# 705777K

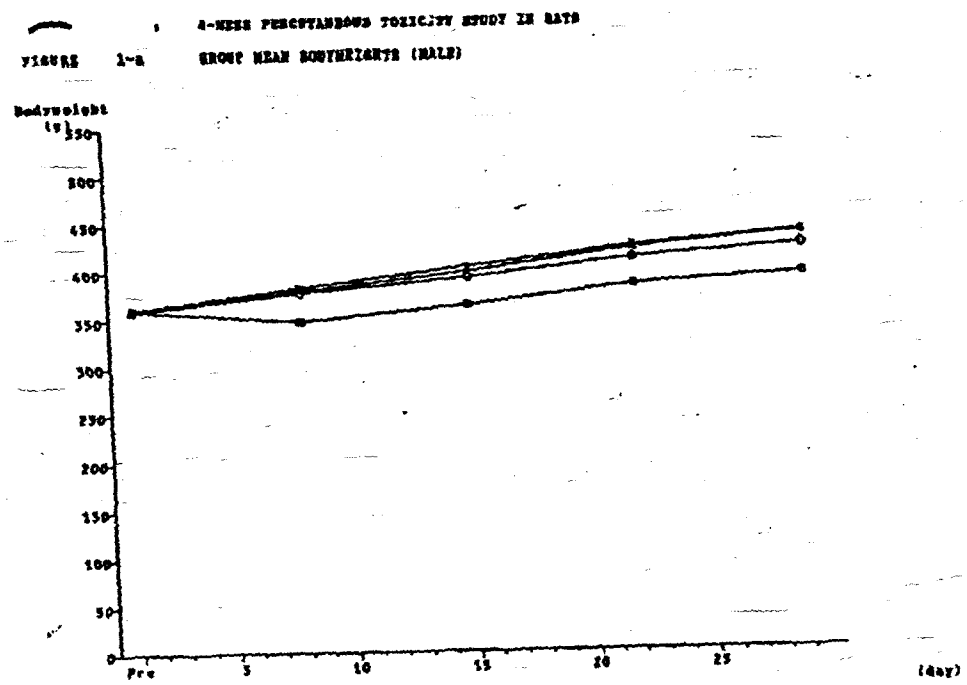
Formulation/vehicle: Same as clinical formulation, described in clinical formulation section previously

Observations and times:

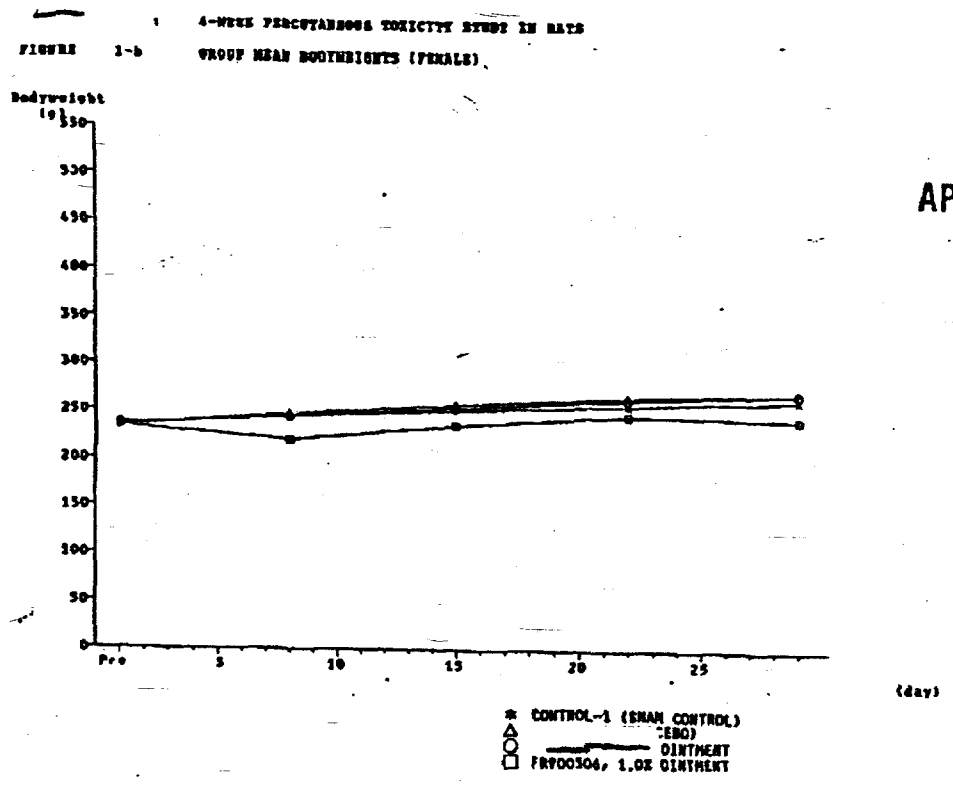
- *Clinical signs:*	twice daily
- *Local dermal signs:*	prior to dosing on days 1, 2, 3, 4, 8, 15, 22 and 29 according to the Draize scale
- *Body weights:*	weekly
- *Food consumption:*	weekly
- *Ophthalmoscopy:*	prior to treatment and during week 4
- *Hematology:*	during week 4
- *Clinical chemistry:*	during week 4
- *Urinalysis:*	during week 4
- *Gross pathology:*	at sacrifice
- *Organs weighed:*	heart, brain, thyroids, spleen, pituitary, testes, liver, thymus, ovaries, kidneys, adrenals and uterus
- *Histopathology:*	The following organs were preserved in 10% buffered formalin: heart, femoral bone marrow, mesenteric lymph node, tongue, esophagus, colon, urinary bladder, adrenals, prostate, spinal cord, sternum, skeletal muscle, thoracic aorta, sublingual gland, stomach, rectum, testes, ovaries, eyeball, femur, sternal bone marrow, thymus, trachea, duodenum, liver, pituitary, epididymides, uterus, optic nerve, skin, sciatic nerve, mandibular lymph node, brochus, submandibular gland, jejunum, pancreas, thyroids, seminal vesicle, vagina, harderian gland, mammary gland, dosing site (dorsal skin), spleen, lung, ileum, kidneys parathyroids and brain.
	All tissues and organs were examined for all treatment groups.

Results:

- **Clinical signs** — No treatment related deaths or clinical signs were noted in this study.
- **Local dermal signs** — No treatment related dermal effects were noted in this study.
- **Body weights** — Bodyweight in the ———— ointment treated animals increased similarly to that observed in both controls. Bodyweight was significantly decreased in male and female animals in the FR900506 ointment group compared to both controls during the first week of dosing. During the second and third weeks of dosing the bodyweight increased in the FR900506 ointment treated animals. The fourth week measurement noted a decrease in bodyweight in the FR900506 ointment treated animals. Total weight gain of males and females in the FR900506 ointment group was significantly lower than that of both controls. Bodyweight figures for male and female animals are reproduced below from the electronic NDA.



APPEARS THIS WAY ON ORIGINAL



APPEARS THIS WAY ON ORIGINAL

- **Food Consumption** — No difference was noted for food consumption in the —— ointment treated animals compared to control animals. Food consumption was lower in the FR900506 ointment group compared to control groups in week 1. Food consumption in FR900506 ointment treated animals was similar to control groups from week 2 – 4.

- **Ophthalmoscopy** — No treatment related effects were noted during the ophthalmic examination.

- **Hematology** — No significant treatment related hematologic effects were noted in this study.

- **Clinical chemistry** — No treatment related effects on clinical chemistry parameters were noted in —— ointment treated animals compared with control animals. Blood urea nitrogen levels were slightly elevated in male and female rats in the FR900506 ointment group (males: ↑42%; females: ↑40%) compared to control animals. Triglyceride levels were elevated in male and female rats in the FR900506 ointment group (males: ↑91%; females: ↑125%) compared to control animals.

- **Urinalysis** — No treatment related effects on urinalysis were noted in the —— ointment or FR900506 ointment groups compared to control animals.

- **Organ weights** No treatment related effects were noted for organ weights.
- **Gross pathology** No treatment related effects were noted during the gross pathology examination.
- **Histopathology**

No treatment related histopathological effects were noted in the —— ointment treated animals.

Treatment related effects were noted in the kidneys of FR900506 ointment treated animals. Minimal basophilic tubules was noted in 1/10 males and minimal outer medullary calcification of the kidney was noted in 2/10 males and 1/10 females.

Treatment related effects were noted in the thymus of FR900506 ointment treated rats. Minimal to moderate cortical thickening with medullary atrophy in the thymus was noted in all the males and 9/10 females.

Treatment related effects were noted in the mandibular lymph nodes of FR900506 ointment treated rats. Mild decrease of the germinal center was noted in 6/10 males and 5/10 females.

Treatment related effects were noted in the pancreas of FR900506 ointment treated animals. Minimal vacuolation of the islets in the pancreas was noted in 4/10 males and 1/10 females.

Key Study Findings:

Potential target organs consisting of kidney, lymph nodes (mandibular), thymus and pancreas were identified in this study for the FR900506 ointment treated animals. Target organs identified in this study are consistent with the pharmacologic effect (immunosuppressant) of tacrolimus. No toxicity was noted in the ——ointment treated animals after 4 week repeat dose administration in rats. This result suggests that the ——impurity does not contribute to the toxicity profile of the FR900506 ointment.

Degradation Product Toxicology Study #4:

Note: The two following genotoxicity studies were submitted to IND ——— Serial #051 (submitted 2/5/97). The final study reports for these two studies were not submitted to the NDA. The review for these two studies are reproduced below from the review of the studies submitted to the IND.

Mutagenicity study of ——— Reversion test with Bacteria

Study Title:	Mutagenicity study of ——— Reversion test with Bacteria
Study Number:	R96-0136-506-P2-E
Performing Organization:	Fujisawa Pharmaceutical Co., Japan
Date Study Completed:	12-96
Drug Lot Number:	#725067L
Tested in:	*Salmonella typhimurium* strains TA98, TA100, TA1535 and TA1537 and *Escherichia coli* strain WP2 *uvrA* in the presence and absence of exogenous metabolic activation (microsomal S9 fraction)
Concentrations tested:	39.1 - 5,000 µg/plate — without S9 mix and 156 - 5,000 µg/plate — with S9 mix. — was dissolved in DMSO and DMSO was used as a solvent control. The appropriate positive control mutagens were run for each tester strain
GLP Study:	No

Under the conditions of this study, the test substance was not mutagenic in the Ames test, with or without metabolic activation, at doses ≤ 5 mg/plate.

Degradation Product Toxicology Study #5:

Mutagenicity study of ——— chromosome aberration test with Chinese hamster lung cells in culture

Study Title:	Mutagenicity study of ——— - chromosome aberration test with Chinese hamster lung cells in culture
Study Number:	R96-0133-506-P2-E
Performing Organization:	Fujisawa Pharmaceutical Co., Japan
Date Study Completed:	12-96
Drug Lot Number:	#725067L
Tested in:	Chinese hamster lung fibroblast cells in the presence and absence of exogenous metabolic activation (microsomal S9 fraction)
Concentrations tested:	25, 50 and 100 µg/ml ——— (for 24 and 48 hr treatments) were tested in the non-activated system. There was 51% cytotoxicity expressed after 24 hr and 62% cytotoxicity expressed after 48 hr at the 100 µg/ml concentration. 50, 100 and 200 µg/ml ——— (incubated with test article for 6 hours, washed, and then incubated for an additional 18 hours) were tested in the activated system. There was 76% cytotoxicity expressed at the 200 µg/ml concentration in this system. Appropriate positive control compounds were run for each assay condition (0.04 µg/ml mitomycin C for the non-activated system and 500 µg/ml dimethylnitrosamine for the activated system). The test articles were dissolved in DMSO and DMSO was used as a solvent control
GLP Study:	No

Under the conditions of this study, the test substance was not clastogenic in the chromosomal aberration assay, with or without metabolic activation.

Summary of Degradation Product Studies:

The sponsor determined that —— expressed similar acute intravenous toxicity as FR900506 in rats, was a non-irritant at —— concentration in rabbits, was not mutagenic in the Ames test or clastogenic in the chromosomal aberration assay. Even though the dermal irritation, Ames test and chromosomal aberration assay were not conducted under GLP conditions, they were conducted under conditions with well documented SOP criteria. Therefore, I believe that the results from these experiments represent results that can be used to assess the toxic potential of the —— impurity in FR900506 ointment.

The sponsor also conducted a 4 week repeat dermal toxicity study in rats comparing the toxicity profile of —— ointment and 1% FR900506 ointment to vehicle ointment and sham control animals, per the division's request. In this study the 1% FR900506 exhibited a toxicity profile consistent with what has been observed in previous studies. However, no toxicity was noted in the —— ointment treated animals in this study. This result suggests that the —— impurity does not contribute to the toxicity profile of the FR900506 ointment.

The results from the 5 toxicity studies conducted with the ——impurity serve to qualify the —— impurity in the FR900506 ointment.

Genetic Toxicology Studies (data from NDAs 50-708/50-709):

The following information is contained in the Prograf® label:

No evidence of genotoxicity was seen in bacterial (Salmonella and E. Coli) or mammalian (Chinese hamster lung-derived cells) *in vitro* assays of mutagenicity, the *in vitro* CHO/HGPRT assay of mutagenicity, or *in vivo* clastogenicity assays performed in mice; tacrolimus did not cause unscheduled DNA synthesis in rodent hepatocytes[37,38,39,40,41,42,43,44].

---

[37] Mutagenicity study of FR900506 – reversion test with bacteria. (1988) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR880249 (R88-0052-506-P2-E).

[38] Mutagenicty study of FR900506 – reversion test with bacteria (II). (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR910396 (R91-0022-506-P2-E).

[39] Mutagenicity study of FR900506 – chromosomal aberration test with Chinese Hamster Lung cells in culture (1988) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR880250 (R88-0050-506-P2-E).

[40] Evaluation of FR900506 in chromosomal aberration test with Chinese Hamster Lung cell line V79 in culture (II). (1993) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR930182 (R93-0019-506-P2-E).

[41] Evaluation of FR900506 in the CHO/HGPRT gene mutation assay. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R91-0069-506-P2-E).

[42] Mutagenicity study of FR900506 – micronucleus test in mice (single oral dosing). (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR910320 (R91-0025-506-P2-E).

[43] Mutagenicity study of FR900506 – micronucleus in male and female mice (1993) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR930076 (R93-0074-506-P2-E).

Carcinogenicity Studies (data from NDAs 50-708/50-709):

The following information is contained in the Prograf® label:

An increased incidence of malignancy is a recognized complication of immunosuppression in recipients of organ transplants. The most common forms of neoplasms are non-Hodgkin's lymphomas and carcinomas of the skin. As with other immunosuppressive therapies, the risk of malignancies in Prograf recipients may be higher than in the normal, healthy population. Lymphoproliferative disorders associated with Epstein-Barr Virus infection have been seen. It has been reported that reduction or discontinuation of immunosuppression may cause the lesions to regress.

Carcinogenicity studies were carried out in male and female rats and mice. In the 80-week mouse study and in the 104-week rat study no relationship of tumor incidence to tacrolimus dosage was found[45,46,47,48,49,50,51]. The highest doses used in the mouse and rat studies were 0.8 – 2.5 times (mice) and 3.5 – 7.1 times (rats) the recommended clinical dose range of 0.1 – 0.2 mg/kg/day when corrected for body surface.

Reviewer Comments: It is important to note that the pharmacokinetic data available from the systemic (oral-feed) carcinogenicity studies referred to in the Prograf label indicated that the bioavailability of tacrolimus from the feed was very poor. This may be a contributing factor for why no tumors were noted in either the rat or mouse systemic carcinogenicity studies.

APPEARS THIS WAY
ON ORIGINAL

[44] Evaluation of the potential of FR900506 to induce unscheduled DNA synthesis in the in vitro hepatocyte DNA repair assay using the male F-344 rat. (1991) Fujisawa Pharmaceutical Co., Ltd.; Company Report GLR910559 (R91-0070-506-P2-E).

[45] FR900506 potential tumorigenic effects in prolonged dietary administration to mice (weeks 1-80). (1993) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R93-0030-506-P2-E).

[46] FR900506 potential tumorigenic effects in prolonged dietary administration to mice (weeks 1-80) (supplement to final report). (1994) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R94-0166-506-P2-E).

[47] FR900506 potential tumorigenic effects in prolonged dietary administration to mice (weeks 1-80) (supplement to final report). (1995) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R95-0075-506-P2-E).

[48] FR900506 potential tumorigenic effects in prolonged dietary administration to rats. (1993) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R93-0055-506-P2-E).

[49] FR900506 potential tumorigenic effects in prolonged dietary administration to rats (supplement to final report) (1194) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R94-0167-506-P2-E).

[50] FR900506 potential tumorigenic effects in prolonged dietary administration to rats (II). (1994) Fujisawa Pharmaceutical Co., Ltd.; Company Report (R94-0165-506-P2-E).

[51] Comprehensive summary of rodent CA studies of FK506 (Phase IV commitments). (1995) Fujisawa USA, In.; Company Report R95-0172-506-P2-E.

Carcinogenicity Studies (submitted to the NDA; initially reviewed under IND ———:

Carcinogenicity Study #1:

Twelve-month photocarcinogenesis study of topically administered FR900506 (FK506, tacrolimus) ointment with ultraviolet radiation (UVR) in hairless mice

Study Title: Twelve-month photocarcinogenesis study of topically administered FR900506 (FK506, tacrolimus) ointment with ultraviolet radiation (UVR) in hairless mice
Study Number: R98-0138-506-P2-E
Volume Numbers: 24 – 26
Test Facility: ———
Study Date(s): 9-9-96 to 9-12-97
Date of Submission: 9-9-99
GLP Compliance: Yes
QA- Report: Yes (X) No ()
Study Type: One year photo co-carcinogenicity study in hairless mice
Species/strain: Crl:SKH1-hr BR (albino hairless) mice

Number of animals per group; age at start of study: 36/sex/group; 38 days old (males: 25 - 35 g, females: 19 - 30 g)

Animal housing: The mice were individually housed in stainless steel cages with a floor area of at least 96.8 cm^2 and a height of at least 12.7 cm.

Drug Lot/Batch number(s): Placebo ointment – lot# 707865K, 710167K
0.03% FR900506 ointment – lot# 707965K, 710367K
0.1% FR900506 ointment – lot# 708065K, 710467K
0.3% FR900506 ointment – lot# 708165K, 710567K
1% FR900506 ointment – lot# 706965K, 713168K, 71616XK

Drug Purity / Stability / Homogeneity: Drug purity ranged from ———
Stability expiration date was December 1997

Dosing Schedule: Five times per week
Irradiation Schedule: Irradiation occurred after test article on Monday, Wednesday and Friday. Irradiation occurred before test article on Tuesday and Thursday.
Duration of Treatment: 40 weeks, followed by a 12 week observation period for the development of tumors

Doses: refer to study design table below

Study Design

Treatment	Volume (µl/mouse/day)	Dose (mg/kg/day)	UVR Dose (MED)	UVR Dose (RBU/week)
Untreated Control	0	0	0.27	600
Vehicle Ointment	50	0	0.27	600
0.03% Tacrolimus Ointment	50	0.75	0.27	600
0.1% Tacrolimus Ointment	50	2.5	0.27	600
0.3% Tacrolimus Ointment	50	7.5	0.27	600
1.0% Tacrolimus Ointment	50	25	0.27	600
Untreated Control	0	0	0.46	1020

Tacrolimus ointment or vehicle was applied to the back of each mouse by syringe and spread across the posterior dorsal skin to a total of 20% of the body surface area. There was no occlusion of the application site, nor were the mice fitted with neck collars. The UVR source was a 6.5 kilowatt xenon long arc water cooled burner (——— The xenon arc was surrounded at a distance of 20 cm by a stationary octagonal metal frame holding one 15 x 15 cm glass filter ——— glass, 1 mm thick) on each facet to attenuate contribution in the UVC range. UVR intensity was monitored on all racks by a customized detector which recorded both intensity and cumulative UVR dose (in Robertson-Berger Units {RBU}).

- *Basis of Dose Selection:*

The sponsor submitted the draft protocol for the photocarcinogenicity study to the division in Serial No. 033 on July 15, 1996. The sponsor received concurrence from the division on the dose selection and protocol for this photocarcinogenicity study. A 13-Week Range-Finding Phototoxicity and Tolerance Study of FR900506 (FK506, Tacrolimus) Ointment with Ultraviolet Radiation (UVR) in Hairless Mice was conducted in support of the photocarcinogenicity study protocol. The results of this study demonstrated that the 3% FR900506 ointment dose was not tolerated well in the study with a body weight loss approaching 10%. A high level of dermal irritation was noted in the 3% FR900506 ointment treated animals that would not have been acceptable for the duration of the photocarcinogenicity study. It was determined that the 1% FR900506 ointment dose would probably be the Maximum Tolerated Dose (MTD) in the Photocarcinogenicity study.

- *Relation to Clinical Use:* The intended route in humans is topical administration.

- *Restriction Paradigm for Dietary Restriction Studies:* No
- *Route of Administration:* Topical
- *Frequency of Drug Administration:* 1X/day
- *Dual Controls Employed:* No
- *Interim Sacrifices:* No

- *Satellite PK or Special Study Group(s):* No
- *Unscheduled Sacrifices or Deaths:* No
- *Deviations from Original Study Protocol:* None

Study Results and Frequency of Monitoring:

- *Clinical Observations:* Twice daily for viability and weekly for clinical observations.

Significant increases in the number of male and female mice with emaciation, cold to the touch and dehydration and a significant increase in the number of male mice with decreased motor activity was observed in the 1.0% Tacrolimus ointment dose group just prior to mice being found dead or moribund sacrificed.

A dose dependent and gender related effect on mortality, which was separate from the impact of moribund sacrifices related to tumor burden, was observed after topical Tacrolimus ointment administration. The number of mice found dead or moribund sacrificed unrelated to tumor burden criteria is presented in the following table.

Tacrolimus (%)	UVR Dose (RBU/week)	# of Males found dead	# of Males moribund sacrificed	# of Females found dead	# of Females moribund sacrificed
None	600	0	1	1	1
0	600	1	2	0	0
0.03	600	6	1	5^{+}	0
0.1	600	4	2	6^{++}	1
0.3	600	12^{++}	4	6^{++}	1
1.0	600	25^{++}	6	12^{++}	5^{++}
None	1020	3	0	2	0

+ - $p<0.05$; ++ - $p<0.001$ when compared to control untreated animals.

Topical administration of the 0.03%, 0.1%, 0.3% and 1.0% Tacrolimus ointment concentrations produced biologically important or significant dose dependent increases in the number of male and female mice that were found dead and/or moribund sacrificed (unrelated to tumor burden criteria) as compared with untreated control mice. The increases in mortality unrelated to tumor burden criteria were greater in male mice as compared with female mice for the 0.3% and 1.0% Tacrolimus ointment dose groups. This result is consistent with what has been observed in repeat dose topical administration of Tacrolimus nonclinical studies in mice.

- *Dermal Observations:* The skin was examined weekly for signs of erythema, flaking, edema, ulceration, skin tumors or other indications of skin toxicity.

Topical administration of the vehicle increased the incidences of mice with cutaneous reactions (grade 2 erythema - both male and female mice; grade 1 flaking - female mice) compared to untreated low UVR control mice. All dose groups of Tacrolimus ointment

demonstrated a similar level of cutaneous reactions as was observed in the vehicle treated mice. This result is consistent with previous nonclinical studies which indicate that the vehicle is a mild to moderate irritant after repeat dose exposure.

- *Body Weight:* Body weights were recorded weekly for the first 13 weeks and then every four weeks.

A significant decrease in body weight was noted for male mice in the 1.0% Tacrolimus ointment dose group. The decrease in body weight correlated with the increase in mortality discussed previously. The body weights were calculated by excluding values for mice that died or were sacrificed moribund. This probably explains the reasons why the week 41 body weight average for the 1% Tacrolimus ointment dose is not significantly different from untreated animals. No treatment related decrease in body weight was noted for female mice in any of the treatment groups.

Body weights (Mean ± SD) for male mice are presented in the following table.

Tacrolimus (%)	Week 1	Week 8	Week 17	Week 25	Week 33	Week 41	Week 49	Week 53
None (Low UVR)	31.2 ± 2.3	36.0 ± 2.6	36.4 ± 2.5	38.1 ± 2.9	38.6 ± 3.0	39.6 ± 3.2	40.7 ± 3.3	42.1 ± 3.6
0 (Vehicle)	31.9 ± 3.2	35.5 ± 2.2	36.5 ± 2.5	37.7 ± 2.6	37.6 ± 2.6	37.9 ± 3.2	41.3 ± 2.8	41.7 ± 3.4
0.03	31.8 ± 2.0	34.3 ± 1.8**	35.6 ± 1.4	36.8 ± 2.1*	36.5 ± 1.8*	38.1 ± 2.4	37.4 ± 3.5**	39.3 ± 3.1
0.1	31.3 ± 2.2	34.0 ± 2.0**	35.4 ± 2.0	36.6 ± 1.8*	36.4 ± 2.0*	38.7 ± 3.3	40.1 ± 2.0	ND
0.3	31.0 ± 2.5	34.4 ± 1.8**	35.6 ± 1.4	36.4 ± 2.5*	35.9 ± 2.8*	36.8 ± 4.8	37.6 ± 3.0*	ND
1.0	31.8 ± 2.2	31.7 ± 2.4**	33.2 ± 3.3**	34.8 ± 2.1**	34.5 ± 2.5**	38.5 ± 1.3	ND	ND
None (High UVR)	31.4 ± 2.0	36.0 ± 2.1	37.9 ± 2.3	38.9 ± 2.4	40.2 ± 2.2	40.4 ± 3.5	ND	ND

ND - Not determined due to mortality.
*- $p<0.05$; ** - $p<0.01$ compared to untreated animals.

Body weights (Mean ± SD) for female mice are presented in the following table.

Tacrolimus (%)	Week 1	Week 8	Week 17	Week 25	Week 33	Week 41	Week 49	Week 53
None (Low UVR)	25.3 ± 2.0	28.8 ± 1.9	29.7 ± 1.9	31.3 ± 2.5	31.4 ± 2.7	33.2 ± 3.0	35.2 ± 3.0	35.4 ± 2.8
0 (Vehicle)	25.4 ± 2.1	28.2 ± 2.0	30.1 ± 2.1	30.9 ± 2.6	32.4 ± 2.9	32.4 ± 3.2	34.2 ± 2.9	35.0 ± 2.7
0.03	25.3 ± 2.2	28.0 ± 2.2	29.2 ± 2.2	30.1 ± 2.5	31.4 ± 2.8	32.0 ± 2.9	35.0 ± 3.0	35.0 ± 3.6
0.1	24.8 ± 1.8	27.3 ± 2.2**	28.6 ± 2.0	29.0 ± 2.7**	30.5 ± 2.4	30.8 ± 2.7**	34.4 ± 2.6	ND
0.3	25.0 ± 1.7	27.7 ± 2.2	29.5 ± 2.6	29.9 ± 3.1	32.1 ± 3.0	32.6 ± 3.8	36.1 ± 5.1	ND
1.0	25.0 ± 2.1	26.9 ± 2.5**	28.4 ± 2.1*	30.5 ± 2.3	29.9 ± 2.6	32.0 ± 2.0	ND	ND
None (High UVR)	24.1 ± 2.1	28.1 ± 2.1	31.3 ± 2.7**	32.4 ± 2.6	33.5 ± 2.5**	35.0 ± 2.3	ND	ND

ND - Not determined due to mortality.
* - $p<0.05$; ** - $p<0.01$ compared to untreated animals.

- *Tumor Development:* The locations, sizes and progression history of skin tumors were recorded manually using a grid location system and separate data sheet for each individual test animals. Skin tumor development was evaluated in terms of Prevalence, Median Tumor Onset, Tumor Amplification Factor and Tumor Yield and groups were statistically compared.

Topical administration of the vehicle enhanced photocarcinogenesis. Median tumor onsets for tumors ≥1 mm (sexes combined) were 42 and 33.75 weeks for untreated and vehicle treated animals, respectively. The enhancement of photocarcinogenesis tended to be greater in male mice, as compared to female mice. The median tumor onsets were 44.5 and 33.5 weeks in the male mice and 42 and 36 weeks in the female mice for untreated and vehicle treated animals, respectively. The reduction in median tumor onset periods was greater for the male mice (11 weeks) than for the female mice (6 weeks).

In female mice, topical administration of the test article either reduced or had no effect on UVR-induced skin tumor production when compared with female mice administered the vehicle only. In male mice, enhancement of photocarcinogenesis was observed in the 0.1%, 0.3% and 1.0% Tacrolimus ointment concentration groups when compared with male mice administered the vehicle only.

The median tumor onset for tumors ≥1 mm is provided in the following table.

Tacrolimus (%)	UVR Exposure (RBU/Week)	Male Mice Median (Weeks)	Female Mice Median (Weeks)
None (Untreated)	600	44.5	42
0 (Vehicle)	600	33.5[c]	36[a]
0.03	600	31.5[c]	44
0.1	600	30[c,1]	42
0.3	600	30[c,2]	34[a]
1.0	600	30.5[c,2]	32[b]
None (Untreated)	1020	28[c,3]	28.5[c,3]

a = $p<0.05$ compared to untreated control; b = $p<0.01$ compared to untreated control; c = $p<0.001$ compared to untreated control.
1 = $p<0.05$ compared to vehicle control; 2 = $p<0.01$ compared to vehicle control; 3 = $p<0.001$ compared to vehicle control.

A drastic vehicle effect on the median tumor onset was observed in this study. In addition, there was a smaller effect of tacrolimus ointment on tumor development beyond the vehicle effect. This was most apparent in the male animals. For female animals, there was actually a protective effect observed at the two lowest doses (0.03% and 0.1% Tacrolimus ointment). The reason for this unclear.

Microscopic examination of the skin sites with gross lesions demonstrated the presence of well-differentiated squamous cell carcinoma, papillomas and keratoacanthomas. No significant difference was observed in the distribution of the squamous cell carcinomas, papillomas or keratoacanthomas among the treatment groups. Malignant lymphoma was observed in the dermis and subcutis of one untreated control (low UVR dose) mouse with gross changes in the skin. Other findings in the skin in the areas with gross observations consisted of a focal hyperplasia of the epidermis or a more diffuse acanthosis and hyperkeratosis with dermal inflammation. The areas described as focal epidermal hyperplasia were discrete areas of epidermal proliferation, which frequently had some atypical cells present. These lesions were still present on a basement membrane and were considered to be hyperplastic and not neoplastic at this time.

- *Pharmacokinetics:* The results presented below are from a 4 week repeat dose pharmacokinetic study conducted in hairless mice to provide pharmacokinetic data for the photocarcinogenicity study. The design of this study was provided in detail earlier in this review. The summary tables are reproduced below.

Pharmacokinetic results (mean) for male mice are presented in the following table.

Treatment	C_{max} (ng/ml)			T_{max} (hr)			$AUC_{(0-20.5)}$ (ng·hr/ml)		
	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26
0.03% FK506 ointment	4.92	10.16	9.63	0*	4	0	39.6	140.2	115.7
0.1% FK506 ointment	32.68	35.81	41.71	2	4	2	287.3	439.2	441.0
0.3% FK506 ointment	109.8	48.89	56.34	0	2	0	1153	572.8	687.5

* - The T_{max} value of 0 is not the conventional 0 hr which refers to prior to drug application. This 0 refers to 132 minutes after dose administration.

Pharmacokinetic results (mean) for female mice are presented in the following table.

Treatment	C_{max} (ng/ml)			T_{max} (hr)			$AUC_{(0-20.5)}$ (ng·hr/ml)		
	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26	Day 1	Day 12	Day 26
0.03% FK506 ointment	6.68	13.88	14.18	0*	2	0	47.25	137.3	156.5
0.1% FK506 ointment	36.24	64.45	75.95	0	2	2	351.6	706.7	848.0
0.3% FK506 ointment	102.0	63.2	122.8	0	0	2	1340	737.1	1136

* - The T_{max} value of 0 is not the conventional 0 hr which refers to prior to drug application. This 0 refers to 132 minutes after dose administration.

Blood levels tended to increase with increased strength of FK506 ointment. Female mice C_{max} and AUC levels were generally slightly higher than male mice C_{max} and AUC levels. C_{max} and AUC levels generally increased from Day 1 to Day 12 and reached a plateau from Day 12 to Day 26.

The results from this study indicate that significant systemic absorption was obtained after dermal application of tacrolimus ointment to hairless mice. The high level of systemic absorption is confirmation that the signs of systemic toxicity noted in this study are due to relatively high systemic exposure to tacrolimus.

Overall Interpretation and Evaluation:

- Adequacy of the carcinogenicity studies and appropriateness of the test model:

The photocarcinogenicity model used for this study is an appropriate animal model for analysis of photo co-carcinogenicity. The dose range study conducted to support the doses selected for this study demonstrated that the high dose should be 1.0% tacrolimus ointment. An MTD was reached in this study based on mortality.

- Evaluation of Tumor Findings:

In this study, topical administration of the vehicle greatly enhanced photocarcinogenesis. The vehicle induced enhancement tended to be greater in male mice as compared to female mice. Topical administration of Tacrolimus ointment had an additional small influence on tumor development beyond the vehicle effect, which was more prevalent in male mice. In fact, in female mice low doses of Tacrolimus ointment (0.03% and 0.1%) had a protective effect on the vehicle enhanced photocarcinogenesis. The reason for this is unclear.

The sponsor states in the submission that the enhancement of UVR induced skin tumor development among mice topically administered the vehicle is not without precedent. In a study conducted to evaluate the modification of skin tumor development in UVR exposed hairless mice by topical administration of emollients, mineral oil (USP) greatly enhanced tumorigenesis[52]. The authors speculated that modification of the optical quality of the skin with resulting enhancement of UVR penetration could account for the increase in UVR induced skin tumors. The components of the vehicle for Tacrolimus ointment (propylene carbonate, white wax, mineral oil, paraffin, white petrolatum) does contain — mineral oil which may be a contributing factor to the enhanced photocarcinogenesis.

Summary Conclusions and Recommendations:

- Acceptability of Study(s) or Overall Testing Approach:

I believe that this study is acceptable, because an MTD was obtained in the study. The mortality noted in the 0.3% and 1% tacrolimus ointment groups was probably due to systemic toxicity elicited by tacrolimus. The overall testing approach for the photo co-carcinogenicity study in the mouse was the division's accepted model for this study.

- Major Tumor Findings:

Topical administration of the vehicle enhanced photocarcinogenesis. Median tumor onsets for tumors ≥1 mm (sexes combined) were 42 and 33.75 for untreated and vehicle treated animals, respectively. The enhancement of photocarcinogenesis tended to be greater in male mice, as compared to female mice. The median tumor onsets were 44.5 and 33.5 weeks in the male mice and 42 and 36 weeks in the female mice for untreated and vehicle treated animals, respectively. The reduction in median tumor onset periods was greater for the male mice (11 weeks) than for the female mice (6 weeks).

In female mice, topical administration of the test article either reduced or had no effect on UVR-induced skin tumor production when compared with female mice administered the vehicle only. In male mice, enhancement of photocarcinogenesis was observed in the 0.1%, 0.3% and 1.0% Tacrolimus ointment concentration groups when compared with male mice administered the vehicle only.

[52] Kligman, LH and Kligman, AM. (1992) Petrolatum and other hydrophobic emollients reduce UVB-induced damage. Journal of Dermatological Treatment 3: 3-7.

Microscopic examination of the skin sites with gross lesions demonstrated the presence of well-differentiated squamous cell carcinoma, papillomas and keratoacanthomas. No significant difference was observed in the distribution of the squamous cell carcinomas, papillomas or keratoacanthomas among the treatment groups.

- Non-neoplastic Findings:

A dose-dependent increase in mortality and corresponding decrease in body weight was noted in this study. Vehicle and tacrolimus ointment treated animals exhibited cutaneous reactions consistent with a mild to moderate irritant. No dose-response relationship was noted for the cutaneous reactions, which is consistent with previous results noted in nonclinical dermal toxicity studies conducted in rodents after repeat dose administration.

- Biological Significance:

The decreased time to formation of skin tumors after UV exposure is biologically significant. This result indicates that tacrolimus ointment acts as a photo co-carcinogen. This result is probably due vehicle effect and to the pharmacologic effect of tacrolimus (an immunosuppressant).

- Potential Clinical Implications of Findings:

Due to the significant enhancement of photocarcinogenesis observed with vehicle alone and with the tacrolimus ointment, it was recommended that information be included in the Investigator Brochure as a safety measure for patients using this drug product. The sponsor adequately described the results of the photocarcinogenicity study in the Investigators Brochure. At the end of this description, the sponsor included the following sentence. "The relevance of these findings to humans is not known." I recommended that additional wording be added after this sentence. The wording that was agreed upon by the sponsor and the division is reproduced below.

"However, potential similarities exist between human and animal mechanisms of photocarcinogenicity. Therefore, even though the biologic significance of these results to humans is not clear, patients should minimize or avoid exposure to natural or artificial sunlight."

A similar type warning will be recommended to be included in the labeling for tacrolimus ointment. This will be discussed in more detail in the labeling section below.

- Recommendations for Further Analysis:

No recommendations for further analysis at this time.

Carcinogenicity Study #2:

Topical oncogenicity study of FR900506 (FK506, tacrolimus) ointment in B6C3F1 mice following daily administration for 24 months

Study Title: Topical oncogenicity study of FR900506 (FK506, tacrolimus) ointment in B6C3F1 mice following daily administration for 24 months
Study Number: 95-8005

Note: The dermal carcinogencitiy study conducted with tacrolimus ointment was reviewed in more detail in an addendum review and will be summarized below.

No formal dose range study was conducted to support the doses selected in the 2 year mouse dermal carcinogenicity study. The sponsor anticipated that the doses used in this study would include the maximum tolerated dose (MTD). The dose groups tested in the study are provided in the following table.

Study Design

Group	Treatment	Tacrolimus Amount Applied (mg/kg/day)		Number of Main Study Animals		Number of Toxicokinetic Animals	
		Males	Females	Males	Females	Males	Females
1	Sham Control	0	0	50	50	60	60
2	Vehicle Control	0	0	50	50	60	60
3	0.03% Tacrolimus	0.99	1.1	50	50	60	60
4	0.1% Tacrolimus	3.3	3.7	50	50	60	60
5	0.3% Tacrolimus	10	11	50	50	60	60
6	1.0 % Tacrolimus	37	40	50	50	60	60
7	3.0% Tacrolimus	116	119	50	50	60	60

Group 1 animals received no application of test article but were subjected to all handling procedures. FR900506 ointment and vehicle (100 µl/mouse) were applied daily, seven days per week, for up to 105 consecutive weeks to an area equal to 40% of the estimated total body surface. The ointment from each application remained on the skin until the next application. The FR900506 ointment or vehicle control was applied to the test area by syringe at 2 µl/cm^2 and spread evenly with a rod. The treatment area was unabraded and unoccluded. Prior to the application of each dose, residual test article wiped off with gauze moistened with water and the test area was blotted dry. The treatment area was clipped free of hair on an as needed basis.

High levels of mortality were exhibited in the 0.3%, 1.0% and 3.0% tacrolimus ointment dose groups. All animals died by week 26 in the 3.0% dose group and by week 46 in the 1.0% dose group. Approximately 85% of animals died by the end of the study in the 0.3% dose group. Adequate numbers for statistical evaluation of tumor incidence were available in the sham control, vehicle control, 0.03% and 0.1% tacrolimus ointment groups. The MTD was identified

as the 0.1% tacrolimus ointment dose based on mortality.

Both the trend and pairwise statistical comparison performed by the agency's biostatistical reviewer demonstrated that the incidence of pleomorphic lymphoma was statistically significant in high dose male (25/50) and female animals (27/50) and that the incidence of undifferentiated lymphoma was statistically significant in high dose female animals only (13/50). No statistically significant elevation in skin tumors was noted in this study.

It has been established in the literature and is stated in the tacrolimus label that an increased incidence of malignancy is a recognized complication of immunosuppression therapy. The most common forms of neoplasm are non-Hodgkin's lymphomas and carcinomas of the skin. It is interesting to note that no carcinomas of the skin were noted in this study even though the immunosuppressant was topically applied and there was significant systemic absorption after topical administration. One possible explanation for this observation is that mice in this dermal carcinogenicity study were not exposed to sunlight (or solar simulated light). The incidence of skin carcinoma is humans that have received immunosuppresant therapy is increased in sun exposed areas of the skin. In addition, human papilloma virus may play a role in human skin carcinoma. Immunosuppresant therapy would decrease the human body's ability to suppress the expression of human papilloma virus and thereby increase the potential for skin carcinoma formation via the human papilloma virus expression.

The highest proposed dose for tacrolimus ointment is the 0.1% concentration. This is the concentration level that was the MTD in the dermal carcinogencity study and the concentration at which a statistical increased incidence of pleomorphic and undifferentiated lymphoma was noted. Human pharmacokinetic analysis in the target population (atopic dermatitis patients) have been conducted with 0.3% tacrolimus ointment (Study 94-0-008) with single and repeat doses (human $AUC_{0\text{-}24\ hr}$ = 27.5 ng·hr/ml after 8 days of repeat dose administration). The results from these studies indicate that human systemic exposure after topical administration of tacrolimus ointment is significantly less than what was observed for the no effect level in the mouse dermal carcinogenicity study. The no effect dose in the mouse dermal carcinogenicity study (0.03%) provided an ~6 fold greater AUC level (189 ng·hr/ml ÷ 27.5 ng·hr/ml) than the AUC seen after maximum exposure in humans with 0.3% tacrolimus ointment application. The dose that lymphomas were observed at in the mouse dermal carcinogenicity study (0.1%) provided an ~20X fold greater AUC (534 ng·hr/ml ÷ 27.5 ng·hr/ml) exposure than the AUC seen after maximum exposure in humans with 0.3% tacrolimus ointment. It is important to note that the increase in lymphomas was noted at a dose in the mouse dermal carcinogenicity study that caused systemic immunosuppression.

Reliable AUC data in humans could be obtained from the 0.3% concentration of tacrolimus ointment. AUC data in humans was not provided for the 0.1% and 0.03% concentrations of tacrolimus ointment in the original NDA submission. Only plasma levels were provided for the 0.1% and 0.03% tacrolimus ointment treatments. Therefore, the safety factor for AUC exposure in the mouse dermal carcinogenicity study could potentially be greater when compared to human AUC data from 0.1% tacrolimus ointment treated patients. Therefore, it is my opinion that human patients would not have a high risk of getting lymphomas under conditions of clinical use for the 0.03% and 0.1% tacrolimus ointment.

However, it is recommended that the tumor findings of this study be included in the label. A comparison of levels of systemic exposure in humans vs systemic exposure in the mouse dermal carcinogenicity study should be included in the label for reference purposes.

The results from this 2 year dermal carcinogenicity study were presented to the Executive CAC on 3/14/00. The Executive CAC recommendations and conclusions are presented below.

Executive CAC Recommendations and Conclusions:

1. The committee determined that the mouse dermal carcinogenicity study was adequate and that there was a strong signal for lymphoma.
2. The committee requested historical background incidence rates for hepatocarcinoma, stromal cell sacroma in the cervix and leiomyoma in the uterus for the strain of mouse used in the dermal carcinogenicity study. A request will be sent to the sponsor for these historical background incidence rates.
3. The committee requested clarification whether it was known if the lymphomas noted in this study were of a B-cell or T-cell origin. A request will be sent to the sponsor to determine if this information is known.
4. The committee strongly recommended that if tacrolimus ointment is approved, then the division should consider strong label warnings for the potential lymphoma risk and photocarcinogenic risk associated with tacrolimus ointment use. Also, the committee recommended that wording be added in the label to indicate that even though no skin cancer was noted in the mouse dermal carcinogenicity study, that there may still be a risk in humans due to the presence of human papilloma virus in humans that was not present in mice. It was noted that the exposure at the NOEL for lymphoma were significantly closer to those produced in human at the recommended dose and that this should be considered in the risk benefit as well as the presence of tumors (still of questionable significance) in the lowest dose tested.

A meeting was conducted with the review team for this NDA to discuss the results of the 2 year mouse dermal carcinogencity study on 3-29-00. The result of this meeting was a letter sent to the sponsor (via FAX on 3-30-00) requesting additional clinical data, clinical pharmacology and nonclinical data to address the potential lymphoma risk possibly associated with the use of tacrolimus ointment. The nonclinical pharmacology/toxicology information that was relayed to the sponsor is reproduced below.

1) It is requested that the sponsor provide historical background incidence rates from the contract lab that conducted the 2 year mouse dermal carcinogenicity study for tacrolimus ointment for the following tumor types:

   a) Liver – Carcinoma
   b) Cervix – Stromal Cell Sarcoma
   c) Uterus - Leiomyoma

2) It is requested that the sponsor clarify whether the lymphomas noted in the 2 year mouse dermal carcinogenicity study conducted for tacrolimus ointment were of a B-cell or T-cell origin, if known.

3) It is recommended that the sponsor conduct a nonclinical study in minipigs, or other suitable species, to determine the concentration of tacrolimus in the regional lymph nodes that drain from the skin after topical tacrolimus ointment application to abraded or irritated skin. The purpose of this study is to determine if the concentration of tacrolimus in regional lymph nodes that drain from the skin is higher than or the same as the level of tacrolimus in the blood after topical administration. This information is necessary for the determination of human risk for lymphoma after topical administration of tacrolimus ointment. It is recommended that the sponsor submit the study protocol for this study to the division for review prior to initiation of the study.

The sponsor was informed by tele-conference (3-30-00) that they would be receiving this FAX. In addition, the sponsor was informed that the concern about lymphoma formation after topical use of tacrolimus ointment in atopic dermatitis patients would be discussed in an August, 2000 Advisory Committee meeting.

Note: The sponsor submitted a response to these questions along with a response to several clinical and clinical biopharmaceutics questions on 6-5-00. A review of this response will be conducted separately for that submission. However, it is prudent to mention the additional data included in this submission pertaining to human AUC data for the 0.1% tacrolimus ointment. The sponsor included in this submission data from ongoing pharmacokinetic trials being conducted in Europe to address the concern for pharmacokinetic information in the patient population. In addition, the sponsor stated that they are in the process of NONMEM modeling of the plasma level data available from the clinical studies conducted under NDA 50-777 to estimate AUC levels in 0.1% tacrolimus treated patients. The sponsor states that these data will be provided in a subsequent submission no later than June 30, 2000.

Two pharmacokinetic studies are currently being conducted in Europe. One in adults (FG-06-22; completed enrollment) and one in children (6-12 years of age) (FG-06-23; enrollment ongoing; interim analyses completed). In the two studies, adult and pediatric patients with active atopic dermatitis were treated with 0.1% tacrolimus ointment over a maximal area twice daily for two weeks. Pharmacokinetic blood samples were obtained on days 1, 4 and 14 in adults and on days 1 and 14 in pediatric patients.

The highest mean $AUC_{0\text{-}12\ hr}$ value observed in the adult study was 10.2 ng·hr/ml on day 4 in the group with 36-60% of body surface area treatment (n=9). This would equal an $AUC_{0\text{-}24\ hr}$ value of 20.4 ng·hr/ml. The no effect dose AUC in the dermal carcinogenicity study is ~9 fold greater that the maximum human AUC obtained in this study (189 ng·hr/ml ÷ 20.4 ng·hr/ml). The AUC for the dose that lymphomas were noted in the dermal carcinogenicity study is ~26 fold greater than the maximum human AUC obtained in this study (534 ng·hr/ml ÷ 20.4 ng·hr/ml).

OVERALL SUMMARY AND EVALUATION:

Introduction:

Tacrolimus is a macrolide immunosuppressant that targets the proliferation of T-cells. The topical formulation developed for this NDA (Protopic® {tacrolimus} ointment) has been developed for the treatment of moderate to severe Atopic Dermatitis. Atopic Dermatitis is primarily a pediatric indication and the duration of treatment is chronic. Tacrolimus had undergone a full set of nonclinical toxicology studies to support the oral formulation of tacrolimus under NDA 50-737/50-738. In addition, the sponsor performed additional nonclinical studies to support the topical formulation of tacrolimus (Protopic® ointment). A brief summary of the significant toxicities and corresponding safety evaluation based on these studies is provided in the following section.

Safety Evaluation:

The systemic toxicity for orally administered tacrolimus has been well characterized previously in nonclinical toxicology studies conducted in rats and baboons under NDAs 50-737/50-738. Potential target organs of toxicity identified in these studies included kidneys, pancreas, thymus, lymph nodes and spleen.

A series of nonclinical special toxicology studies were conducted under NDA 50-777 to support the safety of Protopic® (tacrolimus) ointment. A primary dermal irritation study conducted in rabbits determined that 0.5% and 1% tacrolimus ointments were very weak irritants. The eye irritation potential of the 0.1% and 0.5% tacrolimus ointments was considered to be very weak in the rabbit. Tacrolimus ointment (0.5%) was considered to be non-sensitizer in guinea pigs utilizing the Magnusson and Kligman maximization test protocol. Tacrolimus ointment (0.5%) was considered to be non-photosensitizer in guinea pigs utilizing the Adjuvant and strip photosensitivity test with exposure to UVA radiation only. Topical administration of 0.03%, 0.1% and 0.3% tacrolimus ointment and placebo ointment had no effect on the skin pigmentation of Yucatan miniature swine after eight weeks of twice daily application. The results of a non-GLP study conducted in rats suggested that 0.3% tacrolimus ointment did not induce skin atrophy after 3 weeks of daily treatment.

Repeat dose toxicology studies for topically applied tacrolimus ointment were conducted for a duration up to 26 weeks in rats and 52 weeks in micropigs under NDA 50-777. Topical treatment of rats with tacrolimus ointment for 26 weeks generated similar results noted after oral administration in rats. Target organs of toxicity identified in this study included kidneys, thymus, spleen, pancreas, cervical lymph nodes and bone marrow. Treatment related mortalities were noted in the 0.5% and 0.3% tacrolimus ointment treatment groups. The systemic toxicity effects noted in this study were due to the substantial level of cutaneous absorption noted through rat skin. Tacrolimus blood concentration at 6 hours post application of the 0.5% ointment (10 mg/kg/day) was 4.2 ng/ml and 5.0 ng/ml at week 13 and week 26, respectively. For comparison

purposes, the mean FR900506 blood concentration for a 10 mg/kg/day dose in a 13 week oral (dietary admix) toxicity study in mice was 7 - 8 ng/ml. No treatment related dermal reactions were observed macroscopically at the treated skin sites in this study. Microscopically noted dermal reactions at the treatment site included a non-dose related incidence of epithelial hyperplasia/ancanthosis. The NOAEL in rats administered topical tacrolimus ointment daily for 26 weeks was identified as 0.03% (0.6 mg/kg/day; 1.8 mg/m^2/day) in this study based on the systemic toxicity profile.

Topical treatment of minipigs with tacrolimus ointment for 52 weeks generated much less toxicity than was noted in rats. The reason for this is probably related to significantly less systemic absorption through the skin of the minipig compared to the rat. The level of systemic absorption after topical application of tacrolimus ointment in minipigs more approximates that noted in humans. Treatment related dermal effects were noted in this study that exhibited a similar incidence and severity for vehicle ointment treated and FR900506 ointment treated animals. The macroscopic changes included papules, circular purple ring, hyperpigmentation and hypopigmentation. Most of these changes corresponded microscopically to epidermal acanthosis with hyperkeratosis and perivascular mononuclear cell infiltrates in the papillary dermis consistent with hyperplastic dermatitis. The histopathological findings in the treatment site skin were noted with about the same incidence in vehicle ointment and FR900506 ointment treated animals. Therefore, the dermal effects noted in this study are probably related to vehicle ointment rather that FR900506. The only systemic toxicity noted in this study was a significantly lower body weight in females only (noted after week 19) treated with 3.0% tacrolimus ointment. The NOAEL in male micropigs administered topical FR900506 ointment twice daily for 52 weeks was 3.0% (18 mg/kg/day; 486 mg/m^2/day; Month 6 $AUC_{0\text{-}24\ hr}$ = 185 ng·hr/ml) and for female micopigs the NOAEL was 1.0% (6 mg/kg/day; 162 mg/m^2/day; Month 6 $AUC_{0\text{-}24\ hr}$ = 168 ng·hr/ml) in this study.

The sponsor identified an impurity in the tacrolimus ointment as —— and stated that the level of this impurity was —— The —— impurity has been characterized as ——— in which the ——— from ——— The sponsor has conducted five nonclinical toxicology studies for qualification of the —— impurity under NDA 50-777. The sponsor determined that —— expressed similar acute intravenous toxicity as FR900506 in rats, was a non-irritant at — concentration in rabbits, was not mutagenic in the Ames test or clastogenic in the chromosomal aberration assay. The sponsor also conducted a 4 week repeat dermal toxicity study in rats comparing the toxicity profile of ——— ointment and 1% FR900506 ointment to vehicle ointment and sham control animals, per the division's request. In this study the 1% FR900506 exhibited a toxicity profile consistent with what has been observed in previous studies. However, no toxicity was noted in the ——— ointment treated animals in this study. This result suggests that the — impurity does not contribute to the toxicity profile of the FR900506 ointment. The results from the 5 toxicity studies conducted with the —— impurity serve to qualify the —— impurity in the FR900506 ointment.

The reproductive toxicity of orally administered tacrolimus was evaluated in Segment 1 (rats), Segment 2 (rats and rabbits) and Segment 3 (rats) studies in NDAs 50-737/50-738. Orally (gavage) administered tacrolimus altered reproductive function in female animals and reduced

offspring viability during reproductive toxicity studies with rats and rabbits. Male reproductive behavior was slightly altered in rats and rabbits. The changes in reproductive parameters observed during these studies included increased copulatory intervals, decreased implantation, increased loss of fetuses, fewer births, and smaller litter sizes. No reduction in male or female fertility was evident. Adverse effects in offspring whose mothers received tacrolimus during pregnancy included markedly reduced viability and slightly increased incidence of malformation. Based on the results of these studies, oral tacrolimus was labeled as pregnancy category C. The sponsor did not conduct any nonclinical topical application reproductive toxicology studies under NDA 50-777. Therefore, the pregnancy category will remain C and the information contained in the appropriate section of the label that addresses reproductive toxicity that was included in the Prograf®(oral tacrolimus) will be incorporated into the Protopic® (topical tacrolimus) label. This will be addressed in more detail in the Labeling Review section below.

Under NDAs 50-737/50-738 tacrolimus had undergone testing in a full battery of genotoxicity tests and showed no genotoxic potential. In addition, two oral (feed) carcinogenicity studies in mice and rats have been conducted previously for tacrolimus. The results from these two studies were negative but there is some question as to whether the systemic exposure was adequate in these two studies. The sponsor was requested by the division to conduct a 2 year dermal carcinogenicity study in the mouse to support the tacrolimus ointment formulation. The division also requested conduct of a one year photocarcinogenicity study in hairless mice.

The results of the one year photocarcinogenicity study conducted in hairless mice demonstrated that topical administration of the vehicle greatly enhanced photocarcinogenesis. The vehicle induced enhancement tended to be greater in male mice as compared to female mice. Topical administration of tacrolimus ointment (0.03%, 0.1%, 0.3% and 1.0%) had an additional small influence on tumor development beyond the vehicle effect, which was more prevalent in male mice. A dose dependent increase in mortality unrelated to tumor burden was noted after topical administration of the tacrolimus ointment. Significant systemic absorption was obtained after dermal application of tacrolimus ointment to hairless mice. The high level of systemic absorption is confirmation that the signs of systemic toxicity noted in this study are due to relatively high systemic exposure to tacrolimus.

In the two year dermal mouse carcinogenicity study, high levels of mortality were exhibited in the 0.3%, 1.0% and 3.0% tacrolimus ointment dose groups. All animals died by week 26 in the 3.0% dose group and by week 46 in the 1.0% dose group. Approximately 85% of animals died by the end of the study in the 0.3% dose group. Adequate numbers for statistical evaluation of tumor incidence were available in the sham control, vehicle control, 0.03% and 0.1% tacrolimus ointment groups. The MTD was identified as the 0.1% tacrolimus ointment dose based on mortality. A statistically significant elevation in the incidence of pleomorphic lymphoma in high dose male (25/50) and female animals (27/50) and in the incidence of undifferentiated lymphoma in high dose female animals (13/50) was noted in the mouse dermal carcinogenicity study.

Appropriate information from both the photocarcinogenicity and dermal carcinogenicity studies conducted with tacrolimus ointment is recommended for incorporation into the label.

This is discussed in more detail in the labeling section below.

Clinical Relevance of Safety Issues:

The major clinically relevant safety issues identified from the nonclincal studies conducted with tacrolimus ointment relate to the results of the photocarcinogenicity and dermal carcinogenicity studies. The decrease in time to skin tumor development noted in the mouse photocarcinogenicity study is a very strong signal that tacrolimus ointment can potentially increase the risk of skin cancer from UV exposure in humans. Therefore, it has been recommended in the label below to assure that patients minimize sunlight exposure during the use of tacrolimus ointment.

The significant elevation in the incidence in lymphoma noted in the mouse dermal carcinogenicity study is potentially cause for significant concern. The final decision on whether this is a significant safety concern for use of tacrolimus ointment in humans will be dependent on the extent of systemic exposure that occurs at the maximum recommended human dose. The highest proposed dose for tacrolimus ointment is the 0.1% concentration. This is the concentration level that was the MTD in the dermal carcinogencity study and the concentration at which a statistical increased incidence of pleomorphic and undifferentiated lymphoma was noted in mice. Significant systemic absorption of tacrolimus occurs in mice after topical administration of tacrolimus ointment. Human pharmacokinetic data available from the original NDA submission for tacrolimus ointment provided AUC data for the 0.3% tacrolimus ointment. Only plasma levels were available from the 0.03% and 0.1% tacrolimus ointment concentrations.

The no effect dose in the mouse dermal carcinogenicity study (0.03%) provided an ~6 fold greater AUC level than the AUC seen after maximum exposure in humans with 0.3% tacrolimus ointment application. The dose that lymphomas were observed in the mouse dermal carcinogenicity study (0.1%) provided an ~20X fold greater AUC exposure than the AUC seen after maximum exposure in humans with 0.3% tacrolimus ointment.

During the team review meeting conducted on 3-29-00 to discuss the results of the mouse dermal carcinogenicity study, one of the issues discussed was the need for AUC data for the 0.03% and 0.1% tacrolimus ointment concentrations. The clinical pharmacology reviewers recommended that the sponsor conduct another human pharmacokinetic study to obtain this data directly. In addition, the sponsor may be able to provide a rough estimate of the AUC data from the collected plasma levels obtained in the clinical studies conducted to date with tacrolimus ointment. It was recommended that the sponsor perform such a calculation and submit the AUC data to the division for review. This rough estimate will be useful in providing a more accurate estimate of the safety factor for lymphoma formation after topical application of tacrolimus ointment in humans.

Note: The sponsor provided data from a European clinical pharmacokinetic study in a submission dated 6-5-00. A review of this response will be conducted separately for that submission. Only the human AUC data from the 0.1% tacrolimus ointment treated patients will be discussed here in relation to the dermal carcinogenicity study AUC data.

The highest mean $AUC_{0\text{-}12\ hr}$ value observed in the adult study was 10.2 ng·hr/ml on day 4 in the group with 36-60% of body surface area treatment (n=9). This would equal an $AUC_{0\text{-}24\ hr}$ value of 20.4 ng·hr/ml. The no effect dose AUC in the dermal carcinogenicity study is ~9 fold greater that the maximum human AUC obtained in this study (189 ng·hr/ml ÷ 20.4 ng·hr/ml). The AUC for the dose that lymphomas were noted in the dermal carcinogenicity study is ~26 fold greater than the maximum human AUC obtained in this study (534 ng·hr/ml ÷ 20.4 ng·hr/ml). It is my opinion that human patients would not have a high risk of getting lymphomas under conditions of clinical use for the 0.03% and 0.1% tacrolimus ointment. However, human patients may have a higher risk of developing skin cancer with the use of 0.1% tacrolimus ointment based on the results of the nonclinical photocarcinogenicity assay. Therefore, it is recommended that a warning be included in the label for patients to minimize or avoid exposure to natural or artificial sunlight during the use of 0.1% tacrolimus ointment.

Conclusions:

Based on the nonclinical data available for oral tacrolimus and tacrolimus ointment, my recommendation for NDA 50-777 is that it be approvable from a pharmacology/toxicology perspective provided that the recommended changes in the label discussed in the next section are incorporated into the label.

Labeling Review:

Note: The box warning that is on the Prograf® is not included in the Protopic® label. The first sentence in the Prograf® boxed warning is: "Increased susceptibility to infection and the possible development of lymphoma may result from immunosuppression."

[ ]

The following paragraph was contained in the Prograf® as the first paragraph under the Carcinogenesis, Mutagenesis, Impairment of Fertility section.

"An increased incidence of malignancy is a recognized complication of immunosuppression in recipients of organ transplants. The most common forms of neoplasms are non-Hodgkin's lymphomas and carcinomas of the skin. As with other immunosuppressive therapies, the risk of malignancies in Prograf recipients may be higher than in the normal, healthy population. Lymphoproliferative disorders associated with Epstein-Barr Virus infection have been seen. It has been reported that reduction or discontinuation of immunosuppression may cause the lesions to regress."

[ ]

The entire Protopic® label is inserted below. Comments about the portions that relate to nonclinical pharmacology/toxicology will be inserted directly in the appropriate sections. Recommended sections to be deleted are marked by ~~strikeout~~. Recommended sections to be added are marked by highlight.

WITHHOLD 8 PAGE (S)

Draft

Labeling

/S/

Barbara Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 50-777 (000)
HFD-340
HFD-540/DIV FILES
HFD-540/TOX/JACOBS
HFD-540/PHARM/HILL
HFD-540/MO/LABIB
HFD-540/CHEM/HATHAWAY
HFD-540/PM/WRIGHT
C:/MY DOCUMENTS/WORD/NDAS/NDA50777/50777000.DOC

Concurrence Only:
HFD-540/DivDir/JWILKIN /S/ 8/15/00
HFD-540/PharmTL/AJACOBS /S/
INDs 6/27/00

REVIEW AND EVALUATION OF PHARMACOLOGY/TOXICOLOGY DATA:

KEY WORDS: Immunosuppressant, Atopic Dermatitis, Tacrolimus ointment

Reviewer Name: Barbara Hill
Division Name: Dermatologic and Dental Drug Products
HFD#: HFD-540
Review Completion Date: 9-12-00

SEP 13 2000

NDA number: 50-777
Serial number/date/type of submission: BZ / 8-22-00/ Response to Requested Information
Information to sponsor: Yes () No (X)

Sponsor: Fujisawa Healthcare, Inc.
Parkway North Center, Three Parkway North
Deerfield, IL 60015-2548
(847) 317-8800

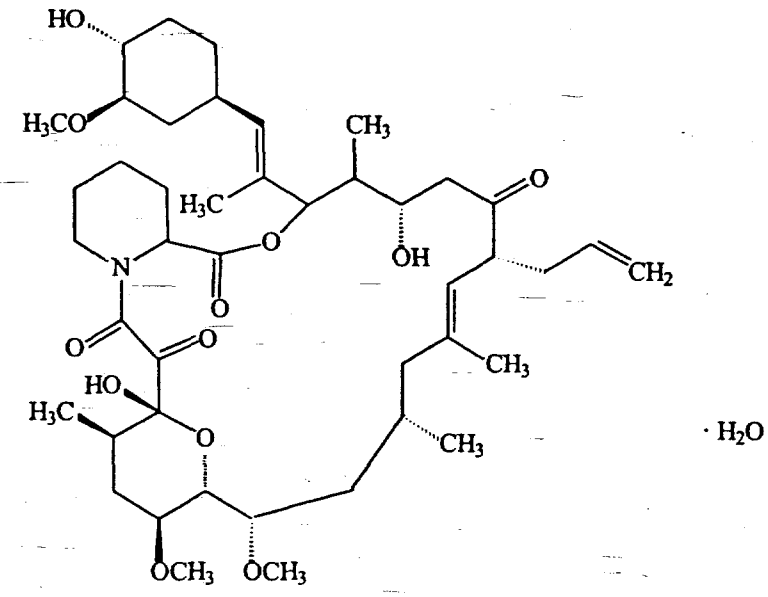
Manufacturer for drug substance: Fujisawa Healthcare, Inc.
3125 Staley Road
Grand Island, NY 14072

Drug:

Code Name: FR900506 ointment
Generic Name: FK-506 ointment
Trade Name: Protopic (Tacrolimus) ointment
Chemical Name: [3S-[3R*[E(1S*,3S*,4S*)], 4S*, 5R*, 8S*, 9E, 12R*, 14R*, 15S*, 16R*, 18S*, 19S*, 26aR*]]-5, 6, 8, 11, 12, 13, 14, 15, 16, 17, 18, 19, 24, 25, 26, 26a-hexadecahydro-5,19-dihydroxy-3-[2-(4-hydroxy-3-methoxycyclohexyl)-1-methylethenyl]-14,16-dimethoxy-4,10,12,18-tetramethyl-8-(2-propenyl)-15,19-epoxy-3H-pyrido[2,1-c][1,4] oxaazacyclotricosine-1,7,20,21(4H,23H)-tetrone, monohydrate
CAS Registry Number: 104987-11-3
Molecular Formula/ Molecular Weight: $C_{44}H_{69}NO_{12} \cdot H_2O$ / 822.05
UV Absorption: λ_{max} (1:1,000 dil in methanol): – nm

APPEARS THIS WAY
ON ORIGINAL

Structure:



Relevant INDs/NDAs/DMFs:

1) NDA 50-708 (Prograf capsules for prophylaxis of organ {liver} rejection; HFD-590)
2) NDA 50-709 (Prograf injection for prophylaxis of organ {liver} rejection; HFD-590)
3) IND [redacted]
4) IND [redacted]
5) IND [redacted](Tacrolimus ointment for Atopic Dermatitis; HFD-540)

Drug Class: Macrolide immunosuppressant

Indication: Moderate to severe Atopic Dermatitis

Dose:

The proposed dose in adults is 0.1% and in pediatric patients is 0.03% [redacted]

Route of administration: Topical dermal

Disclaimer: Note some material may be taken directly from sponsor's submission.

APPEARS THIS WAY
ON ORIGINAL

Introduction and drug history:

A tele-conference was conducted with the sponsor on 7-10-00 to discuss some additional information needs for review of the NDA. One goal of the tele-conference was to inform the sponsor that the advisory committee meeting for tacrolimus ointment has been rescheduled from August 16, 2000 to the second week in November, 2000. The reason for this was the major amendment submitted by the sponsor to the NDA that contained pharmacokinetics data from two studies conducted in Europe. In addition, the division shared with the sponsor two concerns for tacrolimus ointment. The two concerns are listed below.

1) Tacrolimus blood concentrations in pediatric patients (2 to 6 years of age), to model safety margins particularly with respect to lymphoreticular disorders.
2) Photocarcinogenicity potential of tacrolimus ointment.

Based on these concerns, the division requested that the sponsor supply the following information to the NDA.

1) Additional PK data for pediatric patients (2 to 6 years)
2) Responses to the concern regarding the potential for photocarcinogenicity following topical application of tacrolimus ointment.
3) Responses to an article in Nature 1999; 397: 530 entitled "Cyclosporine Induces Cancer Progression by Cell-Autonomous Mechanism."

This submission is a response to the information requested during the tele-conference. A Pharmacology/Toxicology review of the sponsor's responses will be supplied in the following section.

Review of Sponsor's Reply to Requested Information:

Each request will be reproduced below followed by the sponsor's reply to the request. This will be followed by the reviewer's comments for the sponsor's response.

Request 1:

Additional PK data for pediatric patients (2 to 6 years).

Sponsor's Response to Request 1:

The sponsor provided information to address the PK request in attachment 1 of the submission. The sponsor states that this document summarizes the information provided in NDA 50-777 and subsequent submissions in response to the Division's questions regarding blood concentrations following topical application of tacrolimus ointment in young pediatric patients (2 to 6 years of age). The sponsor states that they also included individual patient blood concentrations at various timepoints for these young patients that are listed in an index table in attachment 1 of the

submission. The sponsor also provided floppy disks with the data in Excel files per the Clinical Pharmacologists' request during the teleconference.

Reviewer's Comments for Sponsor's Response to Request 1:

The adequacy of this data will be determined by the reviewing Clinical Pharmacologist for this NDA.

Request 2:

Responses to the concern regarding the potential for photocarcinogenicity following topical application of tacrolimus ointment.

Sponsor's Response to Request 2:

The sponsor provided summary information for the photocarcinogenicity concern associated with tacrolimus ointment in attachment 2 of this submission. The sponsor states that the document is a summary of the photocarcinogenicity assessment by the two leaders in the area of photobiology, Drs. [redacted] The sponsor also states that the document provides a description and interpretation of the study in the context of related studies and supporting information.

Reviewer's Comments for Sponsor's Response to Request 2:

The focus of the photocarcinogenicity document contained in attachment 2 of this submission is to provide an argument that the photocarcinogenesis studies currently conducted in hairless mice have not yet provided a basis for estimating risks to man based on a positive result in this nonclinical animal model. The article emphasizes that the majority of the increased photocarcinogenic response noted in the study was due to the vehicle formulation and that there was only a small additional response noted from the tacrolimus ointment in male mice. This is an accurate representation of the results of the photocarcinogenicity study conducted in hairless mice with tacrolimus ointment. The summary document contributes the increase in photocarcinogenic response to a possible alteration in the optical properties of the outer surface of the skin due to the oily nature of the vehicle. The division is in agreement that this may be one potential explanation for the positive response noted in the photocarcinogenicity study.

In the conclusion portion of the document, it is stated that several examples have identified the photocarcinogenicity assay's qualitative relevance to man, but as a risk assessment tool, the model is essentially undeveloped. The document further states that this difficulty is reflected in the regulatory management of photocarcinogenesis findings in this mode, which is usually limited to a cautionary statement in the labeling, indicating that patients under treatment should avoid concomitant exposure to sunlight or other sources of UVR. The division is in agreement that this has been the policy for addressing potential photocarcinogenic concerns for topical drug products. However, the document further states that "Therefore, an enhanced

response in the hairless mouse photocarcinogenicity model should be noted but not considered a predictor of a clinically detectable carcinogenic potential in man." This is not a totally accurate statement. It is my opinion that a positive result in the photocarcinogencity study is a clear signal for the potential of the drug product to elicit a photocarcinogenic response in humans. This is why it is the division's policy to include a warning in the label to address any potential photocarcinogenic concern for a particular topical drug product.

The final sentence of the document contains the authors recommendation for how to handle the positive signal noted in the photocarcinogenicity study conducted with tacrolimus ointment. The entire sentence is reproduced below.

"Thus, we support the language that was proposed by FHI in its proposed draft package insert for Protopic, [
[ ]
which appears consistent with the current regulatory position as referenced above."

The sponsor actually proposed the following sentence in the draft labeling submitted with the NDA, [ ]
[ ] This sentence is almost the same as the one stated in the document except for the addition of [redacted] in the document version. This sentence was placed immediately after the description of the photocarcinogenicity study results in the label. In my review of the label, I recommended that this sentence be deleted from this section per current division policy and incorporated into the clinical section of the label. This recommendation still stands based on the review of the document contained in this submission. In conclusion, the recommendation proposed in this document is acceptable.

Request 3:

Responses to an article to Nature 1999; 397: 530 entitled "Cyclosporine Induces Cancer Progression by Cell-Autonomous Mechanism."

Sponsor's Response to Request 3:

The sponsor states that the information provided in attachment 3 of this submission is in response to the comment from the Medical Reviewer, Dr. Labib, regarding an article in Nature 1999; 397: 530 entitled "Cyclosporine Induces Cancer Progression by Cell-Autonomous Mechanism." The sponsor also states that this document presents their clarification and additional reference material pertinent to the differential effects of clyclosporine as compared to tacrolimus on TGF-β in humans.

Reviewer's Comments for Sponsor's Response to Request 3:

The Nature manuscript indicates that cyclosporine induces phenotypic changes by a cell-autonomous mechanism. It also suggests that cyclosporine can promote cancer progression by a direct cellular effect that is independent of its effects on host immune cells and may be mediated by transforming growth factor β (TGF-β).

The sponsor states that it is important to note that tacrolimus and cyclosporine are not structurally related but are chemically distinct compounds. In addition, although they have some common effects on calcineurin phosphatase activity, there are numerous distinctions between these two compounds.

The sponsor provided several references that examine TGF-β levels after cyclosporine and tacrolimus treatment in humans. The plasma levels of TGF-β have been measured and compared in patients receiving cyclosporine and tacrolimus. Cyclosporine increased TGF-β plasma levels in these patients, which was directly proportional to the cyclosporine trough whole blood concentration. The elevations of TGF-β in cyclosporine treated patients (15.9 ± 1.7 μg/ml) were above those levels found in normal individuals (~9.0 μg/ml). TGF-β levels in tacrolimus treated patients (10.7 ± 1.3 μg/ml) were significantly lower than cyclosporine treated patients (15.9 ± 1.7 μg/ml), were comparable to levels in normal individuals (~9.0 μg/ml) and were not related to tacrolimus whole blood concentration[1]. TGF-β expression has been studied in renal biopsy samples from kidney transplant patients. Biopsy specimens from patients receiving cyclopsorine showed a significantly greater active TGF-β_1 expression (~2.3 fold greater) than biopsy specimens from patients receiving tacrolimus[2].

The sponsor states that the premise of Hojo, et al, (Nature manuscript) that cyclosporine may induce cancer progression by a mechanism which is mediated through TGF-β is not directly applicable to tacrolimus since clearly these two drugs have distinct and difference effects on TGF-β in humans. The sponsor also states that as demonstrated by the distinct and different effects on TGF-β in humans, one cannot assume that findings with cyclosporine can be extrapolated to tacrolimus. The articles submitted by the sponsor appear to support this viewpoint. It appears that unlike cyclosporine, tacrolimus does not appear to increase TGF-β expression in humans.

[1] Hutchinson IV. (1999) The role of transforming growth factor-β in transplant rejection. Transplantation Proceedings, 31 (Suppl 7A): 9S-13S.

[2] Mohamed MAS, Robertson H, Booth TA, Balupuri S, Kirby JA and Talbot D. (2000) TGF-β expression in renal transplant biopsies. 69: 1002-1005.

APPEARS THIS WAY
ON ORIGINAL

/S/

Barbara Hill, Ph.D.
Reviewing Pharmacologist

cc:
NDA: 50-777 (BZ)
HFD-340
HFD-540/DIV FILES
HFD-540/TOX/JACOBS
HFD-540/PHARM/HILL
HFD-540/MO/LABIB
HFD-540/CHEM/HATHAWAY
HFD-540/PM/WRIGHT
C:/MY DOCUMENTS/WORD/NDAS/NDA50777/50777bz2.DOC

Concurrence Only:
HFD-540/DivDir/JWILKIN /S/ 9/12/00
HFD-540/PharmTL/AJACOBS /S/
/S/
for A.J.
9/13/00

APPEARS THIS WAY
ON ORIGINAL